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Snowpack Ion Accumulation and Loss in a Basin Draining to Lake Superior

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Stottlemyer, R. 1987. Snowpack ion accumulation and loss in a basin draining to Lake Superior. Can. J. Fish. Aquat. Sci. 44: 1812–1819.

The objective of this study was to relate winter precipitation ionic inputs, snowpack retention, and change in first-order stream chemistry with spring snowpack melt. During winter 1982–83, measurement of precipitation inputs, snowpack concentration and loading, and streamwater concentration and discharge of Ca^{2+}, K^+, H^+, NO_3^-, and SO_4^{2-} from a 176-ha watershed reveals that only H^+ might be lost from the snowpack before first thaw. Above-freezing soil temperature beneath the snowpack may be a factor in H^+ loss. An initial 1-d thaw resulted in loss of over one third (6 eq·ha^{-1}) of the snowpack Ca^{2+}. Over one half the snowpack load of K^+, H^+, NO_3^-, and SO_4^{2-}, was lost in a subsequent midwinter freeze–thaw period. Snowpack loading of ionic species was reduced by 70–90% before peak spring melting and stream discharge. Ecosystem H^+ retention and biological uptake of NO_3^- further mitigate ionic "pulses" in streamwater. Sulfate discharge exceeds bulk inputs, which suggests significant dry deposition input and little forest soil retention of this anion. The snowpack was relatively small, which limits wider application of these results to the region.

On tente d'établir une relation entre, d'une part, l'apport d'ions provenant de précipitations hivernales, la permanence de la couverture de neige et les variations chimiques dans des cours d'eau de premier ordre et, d'autre part, la fonte printannière de la neige. Au cours de l'hiver 1982–1983, des quantifications de Ca^{+2}, K^+, H^+, NO_3^- et SO_4^{-2} dans l'apport des précipitations et la couverture de la neige et de leur concentration et élimination dans les cours d'eau d'un bassin versant de 176 ha ont révélé que seul le H^+ peut être libéré de la couverture de neige avant la première fonte. Au-dessus du point de congélation, la température du sol sous la neige peut constituer un facteur lié à la libération du H^+. Une fonte initiale d'une journée a entrainé la libération de plus de 33 % (6 eq·ha^{-1}) du Ca^{+2} emprisonné dans la neige. Plus de 50 % de la charge en K^+, H^+, NO_3^- et SO_4^{-2} présents dans la neige ont été libérés au cours d'une deuxième période de gel–dégel mi-hivernale. La charge nivale d'esèces ioniques a étéréduite de 70 à 90 % avant la période de pointe de la fonte printannière et du débit fluvial. La rétention du H^+ dans l'écosystème et la captation biologique de NO_3^- atténuent davantage les «pulsations» ioniques dans les eaux fluviales. L'élimination du sulfate est supérieur aux apports bruts, ce qui porte à croire à un apport significatif par dépôt sec et une faible rétention de cet anion dans le sol de la forêt. La couverture de neige était relativement peu importante; ce facteur limite une plus grande application des résultats à la région globale.

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In the Great Lakes region, precipitation contains increasing levels of H^+, NO_3^-, SO_4^{2-}, and Pb^{2+} in a west to east gradient from Minnesota to Ohio (Armentano and Loucks 1983; Munger and Eisenreich 1983; Glass and Loucks 1986). In contrast, atmospheric inputs of neutralizing cations show little trend across the region (NADP 1985). Streams and lakes located on Precambrian granites and/or inert glacial sands in this region are potentially sensitive to present precipitation inputs (Eilers et al. 1983; Henriksen 1980; Verry 1983).

Proximity to one of the Great Lakes can significantly increase the quantity of winter precipitation (NOAA 1984). Minor increases in elevation can increase snowfall and snowpack considerably (Stottlemyer 1982). Contaminant loads are then greatest in the upper and potentially more sensitive portions of a watershed. This spatial variation in seasonal precipitation, substrate sensitivity, and deposition of H^+ and acid anions causes difficulty when attempting to generalize the environmental impact of acidic deposition.

A major concern in the region has been the impact of solutes released from the snowpack, especially acids, on stream and lake water chemistry and biota during spring runoff (Jeffries et al. 1979; Cadle et al 1984). Several factors can affect the magnitude of this phenomenon. First, biological uptake in the soil or stream of solutes as NO_3^- can mitigate the impact of HNO_3^- (McLean 1981). Second, periodic midwinter thaws coupled with differential solute leaching from the snowpack can significantly reduce levels of ions such as SO_4^{2-} (Glass 1980). The potential for considerable solute release appears especially high with a sequence of freeze–thaw followed by a slow thaw (Colbeck 1981). Third, H^+ in snowpack melt can be exchanged in the surface soils before reaching the stream (Hanson and Stottlemyer 1985). Heat supplied from underlying, unfrozen soils may also promote gradual solute loss from the snowpack.

The purpose of this study was to relate winter precipitation ionic inputs, snowpack retention, and change in first-order stream chemistry with spring snowpack melt.

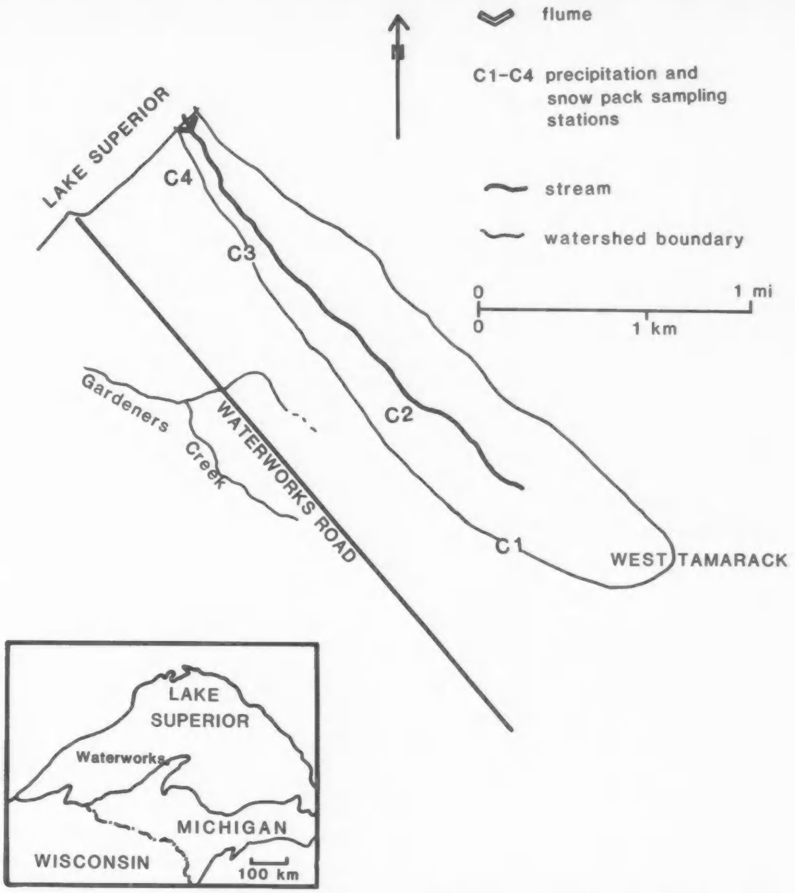

FIG. 1. Calumet Experimental Watershed, Keweenaw Peninsula of Michigan, a 176-ha watershed draining into Lake Superior.

Materials and Methods

In the fall of 1979, hydrological gauging commenced for a small (176-ha) first-order watershed vegetated by 60-yr old sugar maple (*Acer saccharum*), trembling aspen (*Populus tremuloides*), and white birch (*Betula papyrifera*) (Fig. 1). The bedrock is Precambrian metamorphosed Portage Lake Volcanics (basalt and andesite) overlain with glacial drift with a maximum depth of about 10 m. It is adjacent to Lake Superior, has a northwest aspect, and uniform slope varying in elevation from 190 to 375m (mean sea level).

Surface soils were frozen several weeks prior to persistent snowcover. A snowfall on December 28–29 established a permanent snowpack. By January 5, the surface soil temperature was 0.5°C at the uppermost sampling station and never went below this value for the duration of the 1982–83 winter.

To determine precipitation inputs and changes in snowpack in response to elevation, I established four monitoring stations, with the first (C4 in Fig. 1) near the mean elevation of Lake Superior (183 m) and subsequent stations located at approximately 240 (C3), 295 (C2), and 350 m (C1), respectively. Each station was equipped with a bulk precipitation collector (36 cm in diameter). A transect was established at each station where snowpack depth and water content were determined at five locations using a Mt. Rose sampler. Snowpack sampling stations were intentionally located in the open. I measured throughfall in the watershed for two winters and found no significant alteration of incident precipitation chemistry by the hardwood forest canopy (highest probable alteration was for NO_3^-, but $P < 0.653$, unpubl. data). The primary reason for not sampling precipitation and snowpack beneath the canopy was to avoid the possible contamination due to bud scales and bark fragments incorporated in the snowpack which, when melting the snow sample, could contribute to ion concentration.

Samples for determining snowpack quality were collected at each station using a 10-cm-diameter PVC tube. To measure change in the snowpack ion concentration profile, samples were collected vertically throughout the snowpack every 7.5 cm (the resolution of the 500-mL volume snowcutter) at the uppermost (C1) station. Snowpack density and temperature were also recorded.

Stream, snowpack, and precipitation sampling was done weekly on Tuesdays except during periods of rapidly changing stream discharge when a Manning autosampler was used to

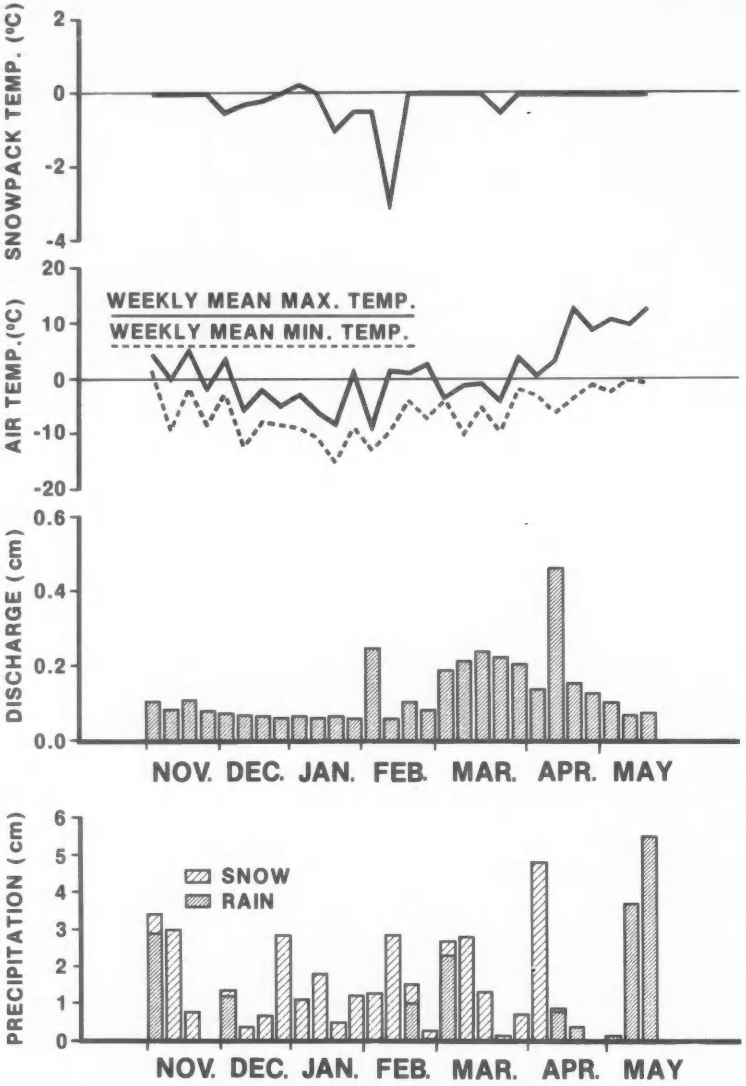

FIG. 2. Precipitation, stream discharge, snowpack temperature 5 cm above soil surface on sampling date, and max./min. air temperature during the period of study in 1982–83, Calumet Experimental Watershed.

collect stream samples every 6 h. Stream discharge was measured using a 30-cm wide Parshall flume equipped with a Stevens recorder. Soil temperature was recorded manually using a thermometer equipped with a stainless steel sensor.

Samples were immediately brought into the laboratory and melted at room temperature in carefully rinsed, covered polyethylene containers. pH, specific conductance, and alkalinity (titration with 0.01 M H_2SO_4 to pH 4.5) were determined as soon as samples reached room temperature. Filtered (0.45 μm) subsamples were refrigerated (2°C) and chemical analyses for Ca^{2+}, Mg^{2+}, Na^+, K^+, NH_4^+, Cl^-, NO_3^-, PO_4^-, and SO_4^{2-} were conducted within 1 wk using ion chromatography (automated Dionex model 10).

Results

Precipitation and Hydrology

Continuous snow cover began the third week of December 1982. Precipitation for the period June 1982 – June 1983 was about average or 88 cm (Stottlemyer 1982). Winter precipitation was dominated (64%) by five large (>2.5 cm of moisture in <30 h) events (Fig. 2) due to southern or southwestern low pressure systems. Storm direction was determined from ground-level wind direction and regional weather maps. Long-term data from the nearby (8 km) Houghton, MI, Federal Aviation Administration weather station indicate that gradual, almost daily precipitation input from lake effects is the more typical condition (NOAA 1984). When storms are from the south or southwest, there is little or no influence of watershed elevation on precipitation amount, and the pattern of cumulative winter precipitation inputs among stations is similar (Fig. 3). But when precipitation is dominated by lake effect from the north or northwest, it increases significantly ($P < 0.0005$) in amount with elevation in the watershed (Stottlemyer 1982).

Most of the cumulative precipitation input was temporarily stored in the snowpack until the middle of February (Fig. 3). The highest mean moisture content of the snowpack occurred in middle January, about 2 mo earlier than I found in winters 1979–86. Peak snowpack moisture content was less than average (Table 1). Midwinter thaws are common. Since the 1979–80 winter to the present the longest period of continual subzero temperatures I have recorded was December 29, 1984, to March 2, 1985.

Stream discharge reached base flow in January (Fig. 2). Above-freezing temperatures in late February and March, a large (2.3 cm) input of rain to the snowpack March 6–9, and mild daytime (mean max. 3.0°C) temperatures during April 1–14 are reflected in the hydrograph. Long-term USGS Hydrologic Benchmark station records for Washington Creek on Isle Royale indicate that spring discharge during April–May usually accounts for 40–60% of the annual discharge (Stottlemyer 1979). U.S. Geological Survey data (Miller et al. 1984) for much larger watersheds (>135 km^2) along the south shore of Lake Superior show values in the 25–35% range.

The stream responds quickly to precipitation events and snowpack melting. This is due to the watershed's medium-textured glacial soils, its small size, and very uniform slope and linear shape (Fig. 1). The hydrograph generally peaks within 1 d following a precipitation event. There was little or no diurnal fluctuation in the March hydrograph. In late April, discharge peaks showed a very consistent and marked diurnal fluctuation, often approaching a 50% increase in discharge from nocturnal rates. This is a response to the higher angle of the sun, the greater diurnal fluctuation in air temperature, and the continued low plant transpiration in April at these latitudes.

Precipitation and Snowpack Quality

Precipitation in the area is acidic (Table 2; Fig. 4). The 4-yr mean weighted H^+ concentration was 28.2 $\mu eq \cdot L^{-1}$ (NADP 1985; Stottlemyer 1982). The cations Ca^{2+}, Mg^{2+}, Na^+, and K^+ were quite uniform in concentration throughout the region (Table 2; Glass and Loucks 1986). Their concentration and that of NH_4^+ declined with snowpack formation in the region (Table 2).

H^+, NO_3^-, and SO_4^{2-} concentrations were intermediate between those observed at NADP stations in Minnesota and lower Michigan (Table 2; NADP 1985; Verry 1983). The NO_3^- concentration was slightly above that observed in Minnesota, but only about one-half that observed in lower Michigan. Other anthropogenic chemical species such as H^+ and SO_4^{2+} were midway in concentration between those observed in Minnesota and lower Michigan. These results are consistent with the findings of others studying the west to east gradient of increasing anthropogenic inputs across this region (Glass and Loucks 1986). H^+ concentration was correlated with NO_3^- ($r > 0$, 99% CI) and SO_4^{2-} ($r > 0$, 99% CI).

I found no significant trends in precipitation quality with increasing elevations above Lake Superior (Bartlett test for homogeneity of group variances). During winter 1982–83, precipitation events from the southeast resulted in a more uniform input among all stations regardless of elevation. Such events, due to their higher concentrations of H^+ and SO_4^{2-}, contributed disproportionately greater deposition inputs. This suggests possible influence on precipitation chemistry from urban industrial areas to the south. Ca^{2+} deposition rates tend to be quite high in the upper midwest due to the extensive exposure of mineral soils in the farming regions to the west and southwest of the Great Lakes (Glass and Loucks 1986). The high Ca^{2+} values in precipitation noted in early April (Fig. 4) probably reflect the rapidly disappearing snowpack in the region.

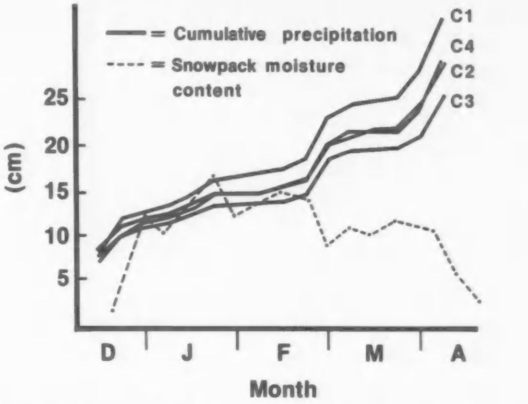

FIG. 3. Cumulative precipitation inputs and mean snowpack moisture content for the sampling stations C1–C4 during period of study in 1982–83, Calumet Experimental Watershed.

TABLE 1. Maximum snowpack moisture content, winters of 1979–86, experimental watershed, Keweenaw Peninsula, MI.

Year	Moisture (cm)
1979–80	33.0
1980–81	24.1
1981–82	33.5
1982–83	19.3
1983–84	29.2
1984–85	30.7
1985–86	47.2

The concentration of H^+ in the snowpack rarely equaled that of precipitation except early in the snowfall period (Fig. 4). Snowpack concentrations peaked in early February and then began a slow decline after the thaw in late February. As in precipitation, the snowpack H^+ concentration was correlated with NO_3^- ($r > 0$, 95% CI) and SO_4^{2-} ($r > 0$, 99% CI).

NO_3^- and SO_4^{2-} concentrations in the snowpack exhibited similar temporal patterns (Fig. 4). NO_3^- concentration was highest in late December, peaked again in late February, and then began to decline in late February concurrent with the decline in snowpack moisture content. Peak snowpack concentration of SO_4^{2-} followed the first rain in early March; then it began to decline but less so than NO_3^-.

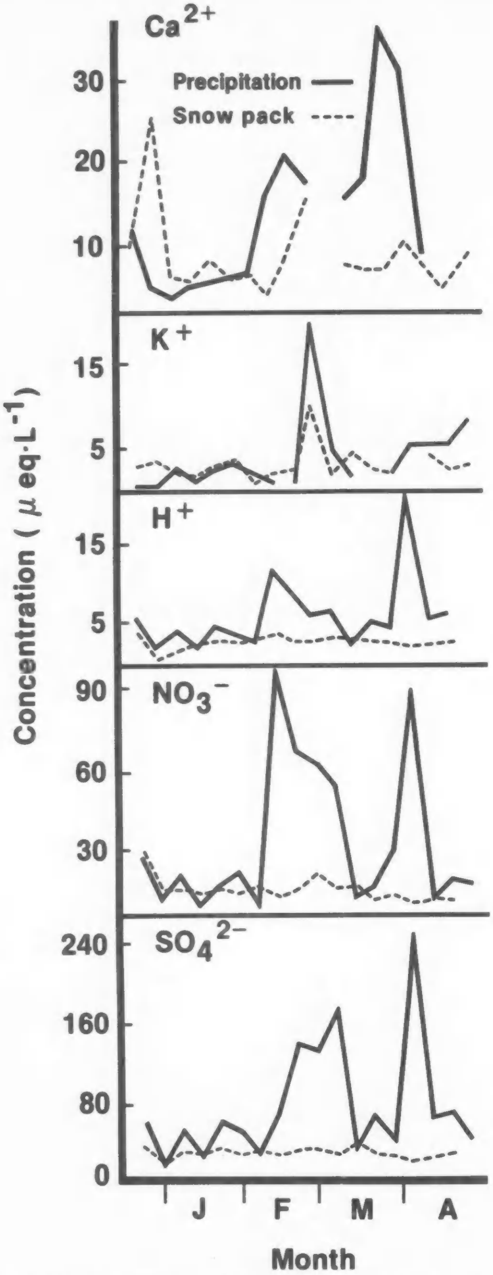

FIG. 4. Mean ionic concentrations for stations C1–C4 for selected chemical species in precipitation and snowpack during period of study in 1982–83, Calumet Experimental Watershed.

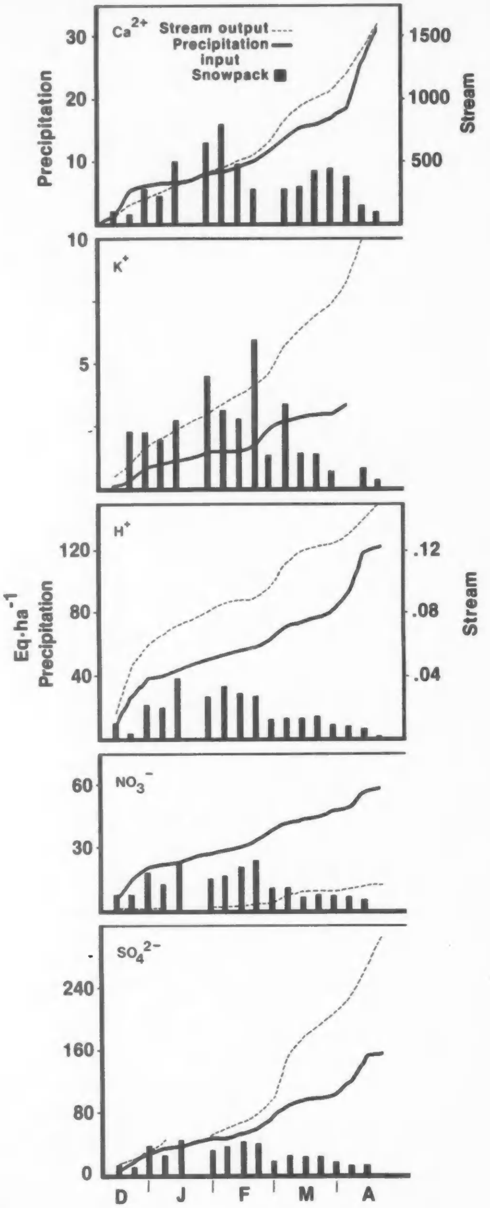

FIG. 5. Weekly average snowpack ionic load, mean cumulative precipitation inputs, and cumulative stream discharge of selected ionic species, Calumet Experimental Watershed.

Snowpack Solute Loads

Snowpack loading and retention of most ion species matched or exceeded bulk precipitation inputs until middle February (Fig. 2 and 5). The Ca^{2+} and K^+ loading in the snowpack exceeded inputs for the first 9 wk (Fig. 5). This might be anticipated for Ca^{2+} due to suspected high dry deposition of this ion in the region prior to permanent snowpack formation (Glass and Loucks 1986). Dry deposition is estimated to be 30–100% of wet precipitation inputs, and an unknown but probably lesser fraction of bulk deposition inputs. During the second week of February there was a very brief thaw during which the Ca^{2+} load in the snowpack declined by 6.5 eq·ha^{-1} (about 40%). From middle February to middle March there was a 4-wk period of

TABLE 2. Mean volume-weighted ion concentrations ($\mu eq \cdot L^{-1}$) for bulk precipitation and snowpack cores (four stations) during period of study, and wet precipitation for selected NADP stations within the region. Chassell, MI, data from NADP station, approximately 16 km from watershed, for winter 1982–83; Fernberg, MN, and Kellogg, MI, data from NADP 1982 annual summary (NADP 1985); Bulk precipitation from bulk collectors located immediately adjacent to snowpack sampling station C1.

Element	Chassell	Fernberg	Kellogg	Bulk precip.	Snowpack
Ca^{2+}	7.2	8.0	12.5	7.7	6.2
Mg^{2+}	1.7	3.1	4.9	2.3	3.4
Na^+	2.9	2.8	2.5	4.8	11.7
K^+	1.3	0.7	0.7	1.4	2.3
NH_4^+	10.7	15.0	21.1	9.6	5.8
H^+	29.0	10.9	48.7	36.5	19.0
NO_3^-	17.9	13.9	30.8	18.7	13.5
SO_4^{2-}	49.7	27.0	61.4	53.9	28.6
Cl^-	0.7	2.3	3.1	1.4	4.2

freezing and thawing, with the Ca^{2+} declining an additional 4 $eq \cdot ha^{-1}$.

The high and variable snowpack loading of K^+ is not so readily explained (Fig. 5). The decline after week 7 is probably due to the input of rain and above-freezing temperatures (Fig. 2), and the increase in late February could be due to the temporary gain in snowpack moisture content from mixed rain and snow (Fig. 2). Some of the variation is probably due to bud scales or other organic debris incorporated in the snowpack. These readily lose K^+ with aging or melting of the snow (Hornbeck and Likens 1973).

H^+ loading in the snowpack never equaled input (Fig. 5). The first brief thaw in the middle of February resulted in a loss of almost one-half the H^+ load in the snowpack. The March 9 precipitation input was largely rain, but the snowpack H^+ content changed little. The snowpack continued its steady loss of H^+ while precipitation inputs increased markedly, especially during April 9–17 (Fig. 5). However, little if any of these inputs and snowpack losses were recorded in stream discharge. Reduced H^+ in snowpacks has also been found in the west (Clements 1966) and east (Hornbeck and Likens 1973). This, however, does not appear to be true in areas where the snowpack was less deep or more dense allowing the soil surface to remain frozen (Jeffries et al. 1979; Jeffries and Snyder 1981).

Very similar patterns of snowpack ion loading were exhibited by NO_3^- and SO_4^{2-} (Fig. 5). The brief thaw during the second week of February resulted in a loss of over one-half of the maximum snowpack load. This resulted in the only pronounced increase in stream discharge of NO_3^- for the study period. During the March 9 rain input, the NO_3^- load in the snowpack changed little (Fig. 2 and 5), indicating that NO_3^- passed through the snowpack. With the final decline of snowpack moisture content in late March (Fig. 3) the snowpack continued its loss of NO_3^-, but the rate of decline was slower than for H^+. As with H^+, little of the NO_3^- input and snowpack losses were recorded in stream discharge (Fig. 5) after the late February increase.

For SO_4^{2-} the February thaw resulted in a snowpack loss of about 30 $eq \cdot ha^{-1}$. During the period there was a precipitation input of about 20 $eq \cdot ha^{-1}$, and together, this resulted in a sharp increase in stream SO_4^{2-} discharge of similar magnitude (Fig. 5). This suggests little or no retention of SO_4^{2-} in the ecosystem, in marked contrast with H^+ and NO_3^-. As with most other ionic species, the March 9 precipitation input of SO_4^{2-} passed through the snowpack (Fig. 5). With the final decline of snowpack moisture content, beginning the last week of March (Fig. 3), the snowpack continued its steady loss of SO_4^{2-} but at a rate much slower than observed for H^+ and more in line with NO_3^+. During the final 4 wk of the study the snowpack lost 20 eq $SO_4^{2-} \cdot ha^{-1}$, precipitation added slightly over 60 $eq \cdot ha^{-1}$, but stream discharge amounted to over 120 $eq \cdot ha^{-1}$ (Fig. 5).

Movement of Ions in the Snowpack

The comparison of total ionic loads in the snowpack relative to deposition is a common way to measure ionic stability in snowpacks. Another method, which perhaps better explains ion dynamics, is to examine the concentration profiles of ionic species in the snowpack throughout winter.

Snowpack Ca^{2+} concentration profiles followed a definite pattern early in the winter, with highest concentrations usually adjacent to the soil (Fig. 6). This pattern was broken shortly after the thaw, which was reflected in the stream hydrograph (Fig. 2), during the second week of February. During this initial period the lowest concentrations were found near or at the surface of the snowpack. This suggests downward movement in the snowpack of this ion to the base where it is retained until the first thaw (Fig. 5). From middle February through the first week in March there was a period of freezing and thawing during which the point of highest Ca^{2+} concentration was found in the upper portions of the snowpack. This suggests loss of Ca^{2+} from the base, and possibly throughout the snowpack. During this 4-wk period, precipitation moisture input was about 5 cm, but the snowpack lost slightly over 4 cm and the stream hydrograph shows this net ecosystem loss (Fig. 2). In the second week of March, following a return to below-freezing air temperatures, the Ca^{2+} concentration profile was similar to that seen in January. By late March, daily maximum air temperatures were consistently above freezing (Fig. 2), precipitation was largely rain, the stream hydrograph continued to climb except for one freezing period April 15–18, and Ca^{2+} concentrations were again highest nearer the surface of the snowpack. The distribution of Ca^{2+} in the snowpack reflects the snowpack's aging. This was verified by snowpack temperature profiles which showed the snowpack to be isothermal from middle February. The high surface concentrations perhaps indicate higher dry deposition resulting from increased dust particles and aerosol

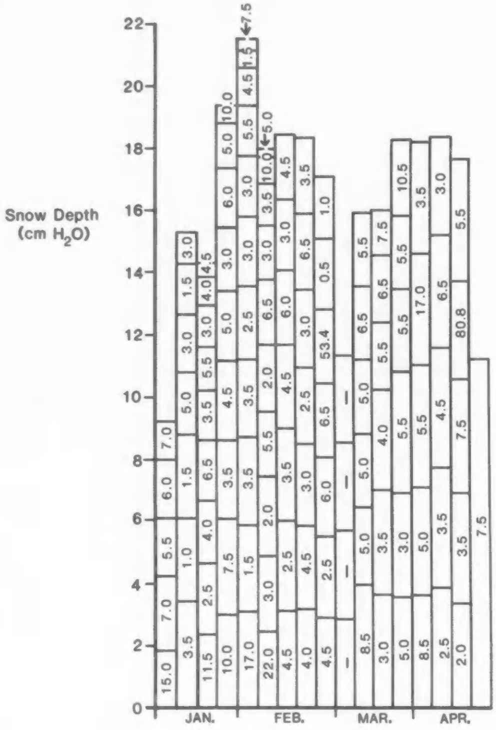

FIG. 6. Weekly Ca^{2+} concentration (μeq) profiles in snow core samples from station C1, Calumet Experimental Watershed.

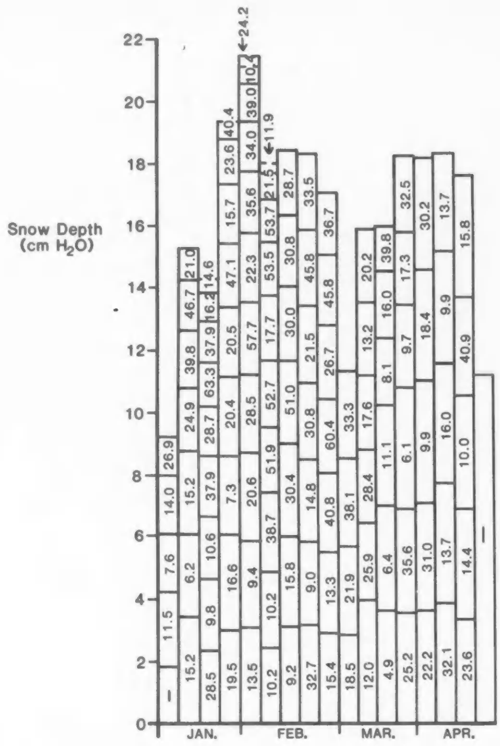

FIG. 7. Weekly SO_4^{2-} concentration (μeq) profiles in snow core samples from station C1, Calumet Experimental Watershed.

formation with the loss of snowpack in the region (Glass and Loucks 1986).

K^+ (not shown) exhibited a similar, if slightly more "noisy," pattern throughout the winter. However, after the middle of March the highest concentration of K^+ was generally not at the surface of the snowpack but nearer its base. As earlier suggested the increased noise with K^+ could be due to dissolution from bud scales or other vegetative materials blown into the snowpack from the adjacent forest.

H^+ and NO_3^- (not shown) exhibited snowpack concentration profiles very similar to SO_4^{2-} (Fig. 7). During the initial 7 wk of the study the highest concentrations were usually in the middle and upper portions of the snowpack, with lower concentrations nearer the base of the snowpack. Middle snowpack values were perhaps the result of the first southerly mixed mist/snow input event (>2.5 cm H_2O) December 25–26 on top of an existing snowpack stabilized by a period of steady below-freezing temperatures (Fig. 2). This made up the first snowpack profile samples (Fig. 6 and 7). After 5 wk, H^+ and SO_4^{2-} showed a decline in the depth of highest ion concentrations. This occurred with initiation of the February thaw period, and especially following the rain in the third week of February. By the following week the snowpack had lost over 50% of its H^+, NO_3^-, and SO_4^{2-} load (Fig. 5). The stream hydrograph continued its steep increase, indicating clearly that the snowpack was aged. With the final decline of snowpack moisture content, beginning the last week of March (Fig. 3), the highest ion concentrations were at or near the base of the snowpack.

Discussion

The distribution of ions within the snowpack before and during a freeze–thaw period and their rapid loss from the ecosystem appear to confirm the laboratory findings of Colbeck (1981) who hypothesized that this type of condition is most conducive to loss of snowpack solutes. Ca^{2+} appeared the most responsive, but within 2 wk of initiating a freeze-thaw period the loading of all ion species declined significantly.

The very mobile K^+ varied most in snowpack concentration and loading. This confirms the findings of Hornbeck and Likens (1973). It is biologically important yet is readily leached from plant tissue, particularly dead tissue such as bark, leaf scales, and grasses.

Earlier studies on Isle Royale (R. Stottlemyer, unpubl. data) showed little influence of hardwoods on winter throughfall quality or quantity. This result was reconfirmed in this study and by others (Mollitor and Raynal 1983). If the study area contained conifers, then the incident winter precipitation and snowpack can be considerably modified both in quantity and quality (Cadle et. al. 1984; Stottlemyer 1982). An ecological factor of potentially greater significance than throughfall is variation in watershed aspect.

The rapid snowpack losses of H^+, NO_3^-, and SO_4^{2-} early in the freeze–thaw period are consistent with the findings of Johannessen and Henriksen (1978), Glass (1980), and R. Stottlemyer (unpubl. data) who melted intact and mixed snowcores in the laboratory.

The ability of the ecosystem to incorporate, by exchange and biological uptake, the entire loads of H^+ and NO_3^- points out the need for holistic examination of the potential consequences of snowpack contaminant loads. This is especially true when one considers the considerable year-to-year variability in snowpack amount, density, and freeze–thaw periods and intensity.

Since there are no known natural sources of SO_4^{2-} in the drainage, the results of this study suggest some temporary ecosystem retention of SO_4^{2-} during the winter in this small watershed. The mechanisms could be many (Reuss and Johnson 1986). SO_4^{2-} is probably in dynamic equilibrium, but it is unlikely that the very same SO_4^{2-} input in the snow is later exported in the stream. My results also indicate the need to look at the question of SO_4^{2-} mobility in forest soils of this region.

It is unfortunate that this was a year of below-average winter precipitation (Table 1). This is clearly a limitation in the present data set. The deviation from normal precipitation input for the January–February period was −4.3 cm (NOAA 1984). During March–April there was a surplus of 4.5 cm above the norm. However, about one half (47%) of this fell as rain.

The results of this study, and the longer term hydrologic data still being collected, clearly demonstrate the considerable risk in drawing general conclusions from short-term studies of something as variable as snowpack formation and its ionic content.

Acknowledgments

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Morphological Divergence and the Organization of Trophic Characters Among Lacustrine Populations of the Threespine Stickleback (*Gasterosteus aculeatus*)

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Lavin, P. A., and J. D. McPhail. 1987. Morphological divergence and the organization of trophic characters among lacustrine populations of the threespine stickleback (*Gasterosteus aculeatus*). Can. J. Fish. Aquat. Sci. 44: 1820–1829.

A multivariate solution for dealing with patterns of character covariance, when character genotypic covariances are known, has been applied to the organization of trophic morphology among lacustrine populations of the threespine stickleback (*Gasterosteus aculeatus*). The quantitative genetics governing eight trophic characters were investigated using representative populations from each of three known trophic ecotypes (open-water, littoral, and intermediate) which occur in the same drainage. Character heritabilities (0.19–0.84) were all significant. Estimates of character genetic correlations ($-0.65 < rG < 0.85$) were obtained and their standard errors were calculated by bootstrapping. Cluster analyses of the genetic correlation matrices defined two character suites, head shape and gill raker structure. Selection gradients between trophic ecotypes indicated that directional selection had operated most strongly on characters related to food size (upper jaw length and gill raker number). These results support the hypothesis that interpopulation variability in trophic morphology is organized as an adaptive response to local selection pressures.

On a appliqueé une analyse multivariée à l'organisation de la morphologie trophique chez des populations lacustres de l'épinoche à trois épines (*Gasterosteus aculeatus*) pour déterminer les schèmes de covariance des caractères quand on connaît les covariances du génotype des caractères. On a étudié les caractères génétiques quantitatifs qui gouvernent huit caractères trophiques chez des populations représentatives provenant de trois écotypes trophiques connus (eau libre, littorale et intermédiaire) présents dans le même bassin versant. Le potentiel héréditaire de chaque caractère (0,19–0,84) était significatif dans tous les cas. On a ensuite obtenu des estimations des corrélations des caractères génétiques ($-0{,}65 < rG < 0{,}85$) et on a calculé leurs écarts types par la méthode «bootstrap». Des analyses typologiques des matrices de corrélation des facteurs génétiques ont défini deux séries de caractères, soit la morphologie de la tête et la structure des branchiospines. Les gradients de choix entre les écotypes trophiques ont révélé qu'un choix directionnel produisait un plus important effet sur les caractères liés à la taille des aliments (longueur de la mâchoire supérieure et nombre de branchiospines). Ces résultats étayent l'hypothèse que la variabilité inter-population de la morphologie trophique représente une réaction adaptative aux pressions de sélection locales.

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The process of reconstructing historical patterns of morphological divergence has been complicated by the observation that continuously varying traits tend to covary (Oxnard 1978; Gould and Lewontin 1979; Arnold 1983; Cheverud 1984; Grant et al. 1985). Characters of evolutionary importance are thought to be largely pleiotropic (Lewontin 1974) and as a result, integrate the phenotype through character correlation (Olson and Miller 1958; Maynard Smith et al. 1985) . Hence, it is necessary to consider groups of covarying characters in studies of adaptive divergence. Recently, this necessity has led to models describing the evolution of correlated characters under directional and stabilizing selection (Lande 1979, 1980; Burger 1986). Lande (1979) has shown that the evolution of a population's mean phenotype under directional selection may be described by the selection gradient

$$\beta = G^{-1}[z(t) - z(0)]$$

where $z(0)$ and $z(t)$ are vectors of character means in the population at times 0 and t and G^{-1} is the inverse of the genetic variance–covariance matrix which is assumed to remain constant over times 0 to t. If G has remained constant, one may consider each element β_i as the net force of directional selection which has acted on each character i to produce the observed changes in population mean morphology. Characters most strongly subject to selection are then readily apparent, which allows the characterization of the selective forces responsible for morphological evolution. This approach has been particularly productive in defining numerous components contributing to the interspecific divergence of the Darwin's finches (Schluter 1984; Price et al. 1984; Price and Grant 1985).

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This is the last of three papers addressing the differentiation of trophic morphology among lacustrine populations of the threespine stickleback (*Gasterosteus aculeatus*), occurring within a single river drainage on Vancouver Island, British Columbia. Our purpose here is to document aspects of the quantitative genetics governing trophic morphology within this system, and having estimated G, to identify individual traits most strongly affected by natural selection over the course of population divergence. Lakes within the drainage contain two well-distinguished ecotypes: a bottom-browsing forager and an open-water pelagic forager. A third trophic ecotype exists which is intermediate in morphology and diet to these two extremes (Lavin and McPhail 1985). Interpopulation variation in trophic morphology produces detectable differences in foraging efficiency between the three types. Foraging ability on a given prey type is consistent with that predicted on the basis of population's diet in the field (Lavin and McPhail 1986); the bottom-browsing morphotype is a superior forager on large, benthic-oriented prey, while the open-water morph is a superior forager on water-column prey. Hence, prey size appears to be a significant selective force organizing interlacustrine variation in trophic morphology for these populations.

If prey size is a significant selective force operating between habitats, morphological traits directly related to handling prey of different size should have been most strongly modified by directional selection. We also investigate the assumption that the genetic variance–covariance matrix (G) has remained constant over the course of population divergence within the drainage.

Materials and Methods

Cowichan Lake

A detailed description of study sites and collecting methods is given in Lavin and McPhail (1985). Samples were collected from a system of lakes contained within the Cowichan River drainage on Vancouver Island (Fig. 1). The upper Cowichan drainage contains Cowichan Lake and four smaller lakes. All the lakes are interconnected and there are no obvious barriers to stickleback dispersal between lakes.

Collections were made at nine sampling sites, from which three trophic morphotypes were identified, each morphotype contained within one of three lake types (i.e. each lake contained only one morphotype). Small, relatively shallow lakes with high total dissolved solids (TDS) and dominated by benthic production contain a bottom-browsing stickleback (Lavin and McPhail 1985) whose diet is composed entirely of macroinvertebrates. The trophic morphology of these populations is characterised by relatively long jaws, permitting a wide gape, and few, short gill rakers (descriptions of these characters are given below). The larger, deeper lakes with lower TDS contain an open-water pelagic forager with numerous long gill rakers and shorter jaws. An intermediate morphotype is also present with jaws like those of the littoral stickleback populations but gill rakers like those of the open-water populations. Sites 1 and 2 contain the bottom-browsing (littoral) morphotype, sites 3 and 5 contain the intermediate morphotype, and all other sites (4, 6–9) contain the open-water morphotype (Fig. 1). A representative population was chosen from each morphotype for our study of the quantitative genetics of trophic morphology (sites 2, 5,and 8; Fig. 1). Collections from the same three sites had been examined previously in the study of functional morphology (Lavin and McPhail 1986).

Establishment and Fostering of Progeny

Mature adults were collected from the three sites in June and July 1984. Parents were chosen without bias from the fish collected at each site. Fertilization of eggs was done on site following the methodology outlined by Bell (1984) but using dechlorinated water transported from the laboratory rather than lake water. This precaution was taken in order to reduce possible effects of lake chemistry on development (e.g. Lindsey 1962). We attempted to make a minimum of 12 crosses from each population; 14 families were obtained for the bottom-browsing population, 12 families for the intermediate population, and 12 families for the pelagic population. Although laboratory mortality was generally low, fungus killed two of the pelagic families. Few breeding adults at this site precluded replacement.

Fertilized eggs were returned to the laboratory and incubated in a water bath at 17.5°C until hatch (approximately 7 d after fertilization). Newly hatched fry were left in the incubator for 3 d and then moved into the light for swimup. After swimup, individual families were placed in 20–L aquaria illuminated by fluorescent lights mounted 20 cm above the tanks. A constant light-dark cycle (16 h light : 8 h dark) was maintained for the entire lifetime of the progeny. Fry were fed an infusoria culture for approximately 1 wk, or until the fry could ingest *Artemia* nauplii. Once the fry attained approximately 20 mm standard length, their diet was switched to a mixture of *Tubifex*, chopped liver, and frozen *Artemia*. Each family, containing 30 or more individuals, was split into two or more subfamilies by removing fish at random and placing them in other tanks. Subdividing families allowed a nested ANOVA to test for effects of common environment which would inflate subsequent estimates of heritability (Falconer 1981). All individuals in all families were killed and preserved after 9 mo.

Progeny and parents were scored for nine characters: standard length (STDLEN), head length (HEAL), snout length (SNOL), eye diameter (EYED), upper jaw length (UPJL), gill raker length (GRL), head depth (HEAD), inner orbital width (INOW), and gill raker number (GRN). All measurements follow Hubbs and Lagler (1958). Standard length of individuals varied widely, within and between populations, introducing a differential growth component to morphological variation. To control for these growth-related differences in size, each variable was adjusted to a standard length of 40 mm. This adjustment uses linear regression of each variable on standard length (Thorpe 1976; Steel and Torrie 1980; Lavin and McPhail 1985). Population-specific regression coefficients were used to adjust each character (Thorpe 1976). All adjusted data were log (base e) transformed before proceeding with any analysis.

Test of Genetic Divergence

A test of divergence among mean morphologies of the three trophic morphs was made to determine whether the observed differences were genetic or environmental. As all individuals from each population were raised under common laboratory conditions, significant differences in phenotypic means must indicate genetic divergence among populations (Mitchell-Olds 1986). Mahalanobis' distances were calculated between popu-

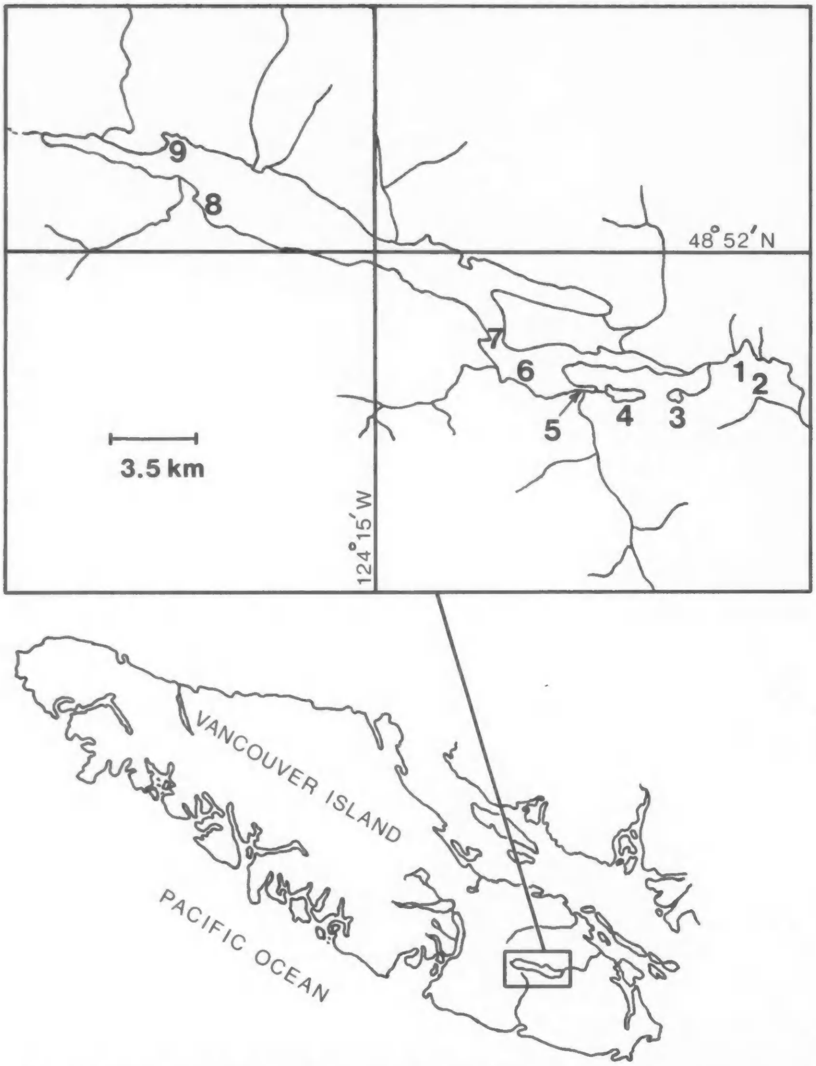

FIG. 1. Cowichan drainage system and the Strait of Georgia region. Sites 1 and 2 contain the littoral morphotype, sites 3 and 5 the intermediate morphotype, and sites 4, 6–9 the open-water morphotype.

lation centroids derived from two separate canonical analyses of discriminance: the first on the wild samples and the second on the laboratory progeny. Significant differences between population centroids were tested by the F ratio (Hope 1968).

Heritability and Genetic Correlation

The "narrow-sense" heritability (h^2) of any polygenic character is defined as the ratio of additive genetic variance $V(a)$ to the character's phenotypic variance $V(p)$ (Falconer 1981). Initially we had hoped to determine h^2 for each character using midparent-offspring regression (Falconer 1981). Unfortunately, there were too few families to obtain reasonable estimates of h^2 using regression; consequently, heritability was estimated from ANOVA using the progeny of the single pair matings (Becker 1975).

In this design, heritability (H^2) is determined from the intraclass correlation coefficient (t) where $t = S^2(a)/S^2(a) + S^2(w)$. $S^2(a)$ estimates the variance among full-sib families, while $S^2(w)$ estimates the variance among individuals. In this instance, H^2 is given as $H^2 \leq 2t$ (Falconer 1981). The inequality results from the estimate of the variance among families; $S^2(a)$ estimates both: one half of the additive genetic variance and one quarter of the dominance variance (Becker 1975). This is the so-called "broad-sense" measure of heritability and is defined as $V(g)/V(p)$ where $V(g) = 1/2V(a) + 1/4V(d) + V(i)$. $V(i)$ is a measure of variance arising from epistatic interaction (Hartl 1980). As a consequence of this measure of H^2, $V(g)$ (the product of heritability and phenotypic variance) sets an upper limit on the magnitude of $V(a)$. Partial dominance, however, does not lead to severe overestimates of $V(a)$, and the contribution of complete dominance will be less than 25% unless the recessive allele is rare (Wright 1952). Heritability and genetic correlations have been estimated from natural populations using full-sib analysis (Arnold 1981; Boag 1983), and these estimates can be very similar to those obtained from more definitive designs (e.g. parent-offspring regression, etc.; Boag 1983).

The estimation of additive genetic covariance between two characters (x,y) proceeds in the same manner as the estimation of genetic variance, but in this instance the variance of a new character $(x + y)$ is partitioned in the ANOVA. The genetic covariance is then

$$\mathrm{Cov}(x, y) = 0.5(V(x + y) - V(x) - V(y))$$

where $V(x)$ and $V(y)$ are the genetic variances and covariances for traits x and y, respectively (Atchley and Rutledge 1980). The genetic correlation (rG) between the traits x and y is

$$\mathrm{Cov}(x, y)/(V(x))^{0.5}\cdot(V(y))^{0.5}.$$

This measure of rG, like H^2, may be inflated by dominance and epistatic effects.

Standard errors of genetic correlations were initially computed following a procedure given by Klein et al. (1973); however, when the genetic correlations exceed the theoretical limits, this procedure yields negative values of the standard error. Therefore the standard error of each estimate was determined from bootstrapping. This method resamples the existing data to estimate the distribution of a statistic when the actual distribution is unknown (Efron 1982). Individual residual scores on a given trait were computed by subtracting each individual's value (X_{ijk}) from its family mean $(\bar{X}_{jk})$ for that trait. Each family (k) was then reconstructed, holding both family means $(\bar{X}_{jk})$ and family size (n_k) constant, by drawing individuals randomly with replacement from the pool of residuals $(X_{ijk} - \bar{X}_{jk})$. A new estimate of the intraclass correlation coefficient was then determined from this bootstrapped data set. This resampling procedure was repeated 300 times for both individual traits (x,y) and pairs of traits $(x + y)$. The standard error of the estimate (H^2 or rG) is the standard deviation of the bootstrapped distribution (Efron and Gong 1983).

Genetic covariance between characters is thought to arise both through linkage and pleiotropy (Hartl 1980). In the latter case, genetic correlations describe the degree to which two phenotypic characters are the same genetic character. Pleiotropy is of particular interest for two reasons. First, patterns of genetic correlation provide insight into character architecture, which allows prediction of correlated responses to selection (Lande 1979; Lande and Arnold 1983). Hence, highly correlated groups of characters may constitute integrated functional units (Olson and Miller 1958; Cheverud 1982) which have evolved together. Second, comparing patterns of genetic correlation between populations allows one to assess the stability of the variance–covariance matrix (G) over the period of population divergence. The constancy of G is a critical assumption of multivariate selection theory discussed below.

Interpreting patterns of single correlations between pairs of characters is hindered by two factors. The first is that single genetic correlations will vary due to differences in gene frequencies, selective regimes, and evolutionary history (Atchley et al. 1981). The second difficulty results simply from the inability to define and describe patterns arising from multiple effects (in this case, 28 genetic correlations within each population); hence, it is preferable to apply some kind of summary technique (Oxnard 1978).

Many authors proceed to extract principal components directly from the matrix of correlations (e.g. Atchley et al. 1981; Boag 1983; Schluter 1984). In this case, character correlations are summarized by component loadings (Pimental 1979), and each new component may be interpreted as a genetically integrated character (Atchley et al. 1981). However, principal components are often ill-suited for use with genetic correlations (Leamy 1977; Boag 1983) ; consequently, we have chosen to summarize patterns of genetic correlations using cluster analysis. In contrast with PCA, cluster analysis is less rigid in its assumptions concerning the nature of the similarity matrix while still defining meaningful groups (Everitt 1974; Leamy 1977). We have used "complete linkage" as the clustering algorithm (Everitt 1974) although other hierarchial, agglomerative techniques have also been used with genetic correlations (Leamy 1977; Boag 1983). Both average linkage and single linkage (Everitt 1974) gave very similar results to those of complete linkage with our data.

Selection Gradients

Historical patterns of population (or species) divergence may be described under certain additive genetic models (e.g. Lande 1979; Burger 1986). Given a knowledge of the genetic covariance between characters, we may weight a particular trait's relative propensity to respond to natural selection by both its own additive genetic variance and its additive genetic covariance with other characters. Clearly, the ability to distinguish those characters most subject to natural selection leads directly to the identification of the selective processes responsible for population change.

The analysis assumes that the Cowichan stickleback populations have diverged from a common marine ancestor. The entire Cowichan valley was completely ice-covered during the last major stade of the Pleistocene glaciation (Fraser stade) approximately 13 500 yr B.P., and the valley was ice-free by about 10 500 yr B.P. (Alley and Chatwin 1979). Electrophoretic patterns from freshwater and marine populations in the Pacific Northwest suggest that anadromous invasions by the marine form of *Gasterosteus* were probably responsible for the recolonization of these deglaciated habitats (Withler and McPhail 1985).

The selection gradient describing the evolution of transition between population mean morphologies is given as

$$\beta = G^{-1}[z(a) - z(b)]$$

where G^{-1} is the inverse of the symmetric matrix of character variances and covariances and $z(a)$ and $z(b)$ are vectors of character means for populations a and b at some time t (Schluter 1984). If the structure of the character complex, as described by G, is constant over evolution, then the net selection gradient is the sum of $\beta = G^{-1}\Delta z$ over the generations 0 to $t - 1$. We have tested the similarity of genetic correlation structure between populations by computing Spearman's rho (r_s, Lofsvold 1986), treating the corresponding elements of each matrix as paired observations. If r_s is negative or close to zero, the patterns of correlation differ between populations. Significance tests were made against Student's t-test (Steel and Torrie 1980).

This net measure of selection (β) is independent of the path taken between $z(0)$ and $z(t - 1)$ and is therefore robust to

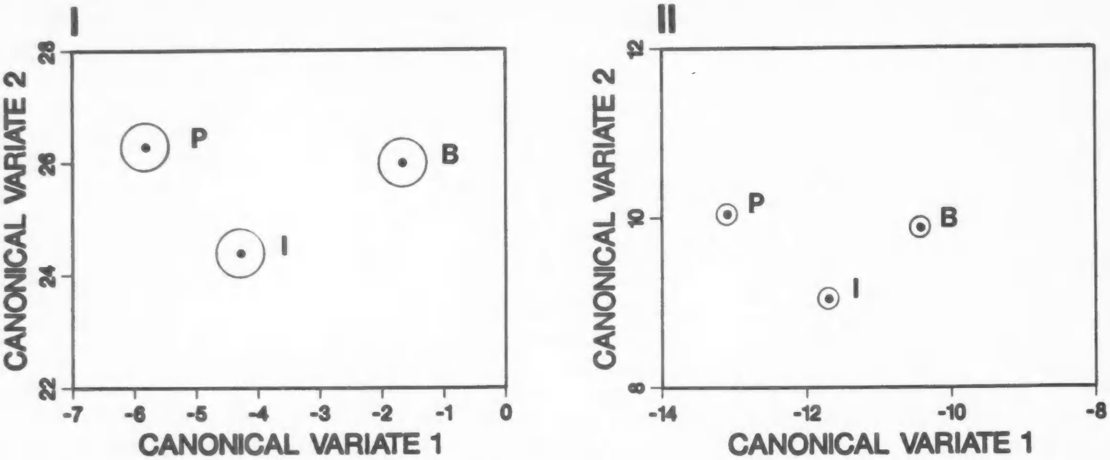

FIG. 2. Population centroids of wild samples (I) and laboratory-reared progeny (II) for three trophic ecotypes of *Gasterosteus* from lakes in the upper Cowichan river drainage. P = open-water; I = intermediate; B = littoral. Circles represent 95% confidence intervals.

changes in the rate and direction of evolution (Lande 1979). The elements β_i of the selection gradient are the net forces of natural selection which have acted on each character independent of the correlated responses to selection on the other characters measured (Schluter 1984). Given the preceding assumptions, we have calculated net selection gradients for population transitions, using the pooled within-populations matrix of genetic variances and covariances.

Results

Test of Genetic Divergence

Morphological differences among ecotypes were preserved under constant laboratory conditions, indicating a genetic component to each trophic trait. Although Mahalanobis' distances between wild sample centroids are greater than for laboratory samples, distances between the latter are highly significant (Table 1). In addition, the relative position of each ecotype in discriminant space remained unchanged between wild and laboratory samples, although the absolute positions are different (Fig. 2). This is due to four more significant character contrasts in the laboratory progeny compared with the wild data (Lavin and McPhail 1986), resulting in slightly different contributions of the measured variables to each canonical axis. These altered contrasts suggest that for populations in the wild, either selection, developmental plasticity, or both are masking some interpopulation genomic divergence.

Heritability and Genetic Correlations

There was no significant contribution of common environment (i.e. tank) to variance between families (ANOVA, $p < 0.05$); hence, individuals were pooled within families before calculation of the intraclass correlation coefficient. All estimates of H^2 were significant for all characters among ecotypes, with the littoral ecotype showing the highest average heritability and the intermediate the lowest (Table 2). Most of the estimates of H^2 are in the low to moderate range ($p < 0.50$), indicating that there is a moderate amount of genetic variance preserved within each population's genome. Because the heritabilities are significant for all characters, we were able to calculate genetic correlations among all possible pairs of traits.

TABLE 1. Mahalanobis' distances between population centroids derived from canonical analysis of discriminance.

	Wild samples			Laboratory progeny		
	D	*F*	*N*[a]	*D*	*F*	*N*[a]
Intermediate/ open-water	5.96	10.28	(30, 30)	2.96	43.75	(357, 176)
Intermediate/ littoral	9.38	16.18	(30, 30)	2.35	46.71	(357, 292)
Open-water/ littoral	17.33	29.88	(30, 30)	7.24	99.59	(176, 292)

NOTE: All pair-wise distances are significant at $p < 0.0001$.

[a] Parenthetical values are sample sizes for each pair of morphotypes.

Character genetic correlations range from 0.01 to 0.84 ($|rG|$); a number of the correlations are negative. Most of the standard errors about each correlation are high, which is a general result for estimates of *rG* (e.g. Van Vleck and Henderson 1961; Arnold 1981; Boag 1983). Standard errors increase both as sample size and the precision of H^2 estimates decrease (Boag 1983), and this is evident from our results. Genetic correlations obtained from the 14 families of littoral progeny show the lowest average standard errors (Table 3a), while standard errors of *rG* increase for the intermediate (Table 3b) and are highest for the open-water ecotype (Table 3c). The larger standard errors of character correlations for the open-water progeny likely arise as a sampling artifact due to small sample size (i.e. family number; Lofsvold 1986) and decreased precision of H^2 estimates. The low precision of genetic correlations is an unfortunate methodological problem, not easily overcome. In some cases, reliable estimates of *rG* may require more than 800 parent–offspring pairs (Van Vleck and Henderson 1961); clearly, the logistics quickly become intractable for the study of multiple populations and characters. For this reason, investigations of genetic correlations operate under the assumption that the observed

TABLE 2. Heritabilities and standard errors for the three representative trophic morphologies. Presented as H^2, based on family size weighted intraclass correlations among full sibs. All estimates are significant at $p < 0.01$. The number of individuals is shown for sample size, with the number of families in parentheses.

	Morphotype		
Character	Intermediate	Open-water	Littoral
HEAL	0.28 (0.076)	0.32 (0.108)	0.24 (0.099)
SNOL	0.22 (0.068)	0.37 (0.122)	0.26 (0.083)
EYED	0.38 (0.086)	0.33 (0.119)	0.84 (0.092)
UPJL	0.21 (0.069)	0.34 (0.117)	0.46 (0.089)
GRL	0.24 (0.073)	0.81 (0.113)	0.23 (0.077)
HEAD	0.19 (0.063)	0.40 (0.122)	0.59 (0.089)
INOW	0.51 (0.082)	0.50 (0.118)	0.53 (0.103)
GRN	0.19 (0.069)	0.30 (0.165)	0.44 (0.091)
N	357 (12)	176 (10)	292 (14)

measures reflect true pleiotropic and linkage arrangements within the population's genome (Atchley and Rutledge 1980; Arnold 1981; Schluter 1984). Results of both animal husbandry studies (Falconer 1981) and investigations of the correlation between phenotype and genotype (Atchley et al. 1981; Cheverud 1982; Boag 1983; Schluter 1984) support this assumption.

The cluster summaries (Fig. 3) of character organization based on *rG*'s defined two major patterns: congruence in character organization between the intermediate and littoral ecotypes and two distinct character associations. Elements of the correlation matrices (*rG*'s) were themselves significantly correlated between the littoral and intermediate populations ($r_s = 0.698$, $p < 0.001$) but neither of these matrices was significantly correlated with the open-water matrix. Considering only patterns of character organization among intermediate and littoral populations for the moment, two character suites are apparent: the first suite groups elements of gill raker morphology (denoted 5 and 8 in Fig. 3), while the second suite contains the measured elements of head shape. Although gill raker number and gill raker length are not tightly correlated in the littoral population compared with the intermediate population, neither character is strongly correlated with the head shape cluster in either population. This pattern contributes to the congruence in *G* between these two populations. Gill raker morphology has been implicated in planktonic foraging in numerous studies (e.g. Kliewer 1971; Magnuson and Heitz 1971; Seghers 1975; Wright et al. 1983); however, the interpretation of the second cluster is not immediately obvious.

We wish to stress that it is the general pattern of character linkage that we are interested in and not individual pairs of character grouping. Minor differences among single genetic correlations (e.g. between gill raker number and gill raker length) may arise from differences in gene frequency and/or selective regime (Atchley et al. 1981). In addition, sampling error and low–precision estimates of *rG* may contribute to differences among populations in patterns of character linkage (Lofsvold 1986). The large standard errors associated with measures of *rG* for the open-water population indicate low precision in this set of results and hence are probably responsible for the different character pattern observed for this ecotype. It is possible that the genetic covariance structure of the open-water population is actually different from that of the other populations. Potential contributions to this difference are multiple invasions and stochastic or selective reorganization of *G*. However, given the lower precision of H^2 estimates for the open-water population, we feel sampling error to be a more conservative explanation of the difference. In contrast, we are confident that the dendrogram summaries of character linkages for the other two populations are more accurate descriptions of pleiotropic relations among characters. Given that these dendrograms summarize the relations among 28 pair-wise genetic correlations, the chance of sampling error alone yielding significant congruence between these two correlation matrices is vanishingly small. Thus, for the littoral and intermediate ecotypes, *G* has remained approximately constant, in contrast with selective and/or stochastic reorganization following an initial founder event in the drainage.

Although gene exchange could maintain similar pleiotropic organization of characters between populations (Price et al. 1984), this is an unlikely source of stability in this case for two reasons. First, populations clearly differ in allele frequencies controlling trophic traits (as evidenced from laboratory crosses), which might be expected to alter genetic correlations. Second, it is unlikely that stickleback move between these two sites (i.e. there is no gene exchange between the littoral and intermediate populations). The two populations are separated by approximately 7 km and an elevational difference of 8 m (Lavin and McPhail 1985), and although there are no physical barriers to movement between these two sites, the freshwater form of *Gasterosteus*, unlike its marine counterpart, does not possess the biomechanical characteristics which contribute to dispersal (Taylor and McPhail 1986).

Selection Gradients

Reconstruction of the net forces of directional selection required for transition between population morphologies requires that *G*, the genetic covariance matrix, has been estimated without error and that it has remained constant (Lande 1979). Because of the low precision of the genetic correlations calculated for the open-water ecotype, we have computed the pooled within-populations genetic covariance matrix from the littoral and intermediate populations only. The congruence of character organization between these two populations supports the assumption of a constant *G*. Minor differences in character genetic correlations do not substantially alter selection gradients, which are fairly robust to interpopulation fluctuations in genetic correlations (Price et al. 1984).

Selection gradients for transitions between the three trophic morphologies are presented in Table 4. The open- water/littoral and open-water/intermediate gradients are weighted most heavily by upper jaw length, gill raker number, and head depth. The total selection required to shift morphologies from either intermediate or littoral to open-water is approximately the same, where total selection is taken to be the sum of the squared values

TABLE 3. Character genetic correlations and their standard errors from laboratory-reared progeny of the littoral, intermediate, and open-water morphotypes. Genetic correlations are given below the diagonal and their standard errors above the diagonal. Standard errors are based on 300 bootstrapped samples.

	HEAL	SNOL	EYED	UPJL	GRL	HEAD	INOW	GRN
				(a) Littoral				
HEAL		0.156	0.146	0.130	0.248	0.189	0.197	0.223
SNOL	0.70		0.159	0.158	0.267	0.177	0.191	0.206
EYED	0.59	0.27		0.077	0.216	0.097	0.101	0.147
UPJL	0.71	0.48	0.79		0.249	0.122	0.134	0.171
GRL	0.27	0.02	−0.04	−0.12		0.204	0.190	0.251
HEAD	0.28	0.29	0.46	0.47	−0.61		0.092	0.146
INOW	0.03	−0.01	0.59	0.52	−0.47	0.68		0.161
GRN	0.39	0.26	0.13	0.38	0.34	−0.34	−0.16	
				(b) Intermediate				
HEAL		0.084	0.179	0.227	0.216	0.239	0.179	0.249
SNOL	0.84		0.211	0.243	0.236	0.227	0.174	0.248
EYED	0.22	0.26		0.157	0.194	0.205	0.129	0.199
UPJL	−0.11	−0.03	0.66		0.235	0.189	0.145	0.229
GRL	−0.07	−0.41	−0.33	0.12		0.254	0.189	0.246
HEAD	0.05	0.33	0.27	0.55	−0.16		0.134	0.221
INOW	−0.06	0.28	0.50	0.65	−0.34	0.73		0.193
GRN	0.01	−0.16	−0.21	−0.20	0.25	−0.65	−0.36	
				(c) Open-water				
HEAL		0.173	0.275	0.211	0.208	0.226	0.232	0.242
SNOL	0.71		0.255	0.244	0.204	0.220	0.250	0.210
EYED	0.37	0.37		0.279	0.174	0.200	0.231	0.270
UPJL	0.58	0.33	0.19		0.192	0.271	0.215	0.318
GRL	−0.17	−0.10	0.51	−0.31		0.140	0.182	0.224
HEAD	0.44	0.55	0.66	0.14	0.62		0.233	0.282
INOW	0.43	−0.22	−0.24	0.49	−0.24	−0.22		0.262
GRN	0.70	0.85	0.33	0.31	−0.19	0.57	−0.15	

of each element in each gradient (Schluter 1984). Less selection would be required to shift morphologies between intermediate and littoral although there has been some selection on gill raker number between these two morphologies.

Discussion

The low to moderate heritabilities measured for our populations provide the potential for an evolutionary response to natural selection. Additive genetic variance is influenced by mutation and selection, gene flow, and drift. It is unlikely that drift is at present significant in reducing additive variance, although it may have been a contributing factor during colonization of the drainage. Lacustrine populations of *Gasterosteus* in the Pacific Northwest typically contain tens of thousands of breeding individuals during the reproductive season, and extremely high densities of reproductive individuals were observed for both the intermediate and littoral populations. In addition, the congruent patterns of character genetic correlations between the intermediate and the littoral populations do not support a drift effect. Different allele frequencies due to sampling error are expected to alter patterns of character covariance which is not apparent for these two populations.

The low heritabilities of the intermediate ecotype were unexpected; rather, we predicted that it should show the highest additive variance, due to both the potential for gene flow with the open-water population and a more heterogenous selective regime than those of the other two populations. The intermediate environment contains well-defined elements of both the open-water and littoral habitats. Adaptive population subdivision (i.e. the niche variation hypothesis; Van Valen 1965) based on these two habitats would preserve additive variance within the entire population. We feel that given the enormous potential for gene flow between the open-water and intermediate ecotypes, differentiation of allele frequencies in the face of gene flow (Endler 1977) is unlikely. Hence, we suggest that for interpopulation genomic divergence to result between the intermediate and open-water populations, some form of selection for habitat choice and/or assortative mating is operating, and that this may have led to the reduced heritabilities observed for the intermediate population.

Selection gradients separating ecotypes indicate that selection has operated most strongly on characters responsible for attaining particles of different size (i.e. upper jaw length and gill raker number). The functional significance of head depth is not known; therefore, we will not attempt to place it a posteriori into an historical narrative. Interpopulation differences in upper jaw length confer differential foraging success on benthic prey (Lavin and McPhail 1986). Increased jaw length allows ingestion of larger prey compared with the open-water population. In addition, benthic foraging success had a behavioural component, independent of intrapopulation variation in upper jaw length, but associated with ecotype. We predict a positive genetic correlation between littoral morphology and behaviour, as this would lead to a superior ability to track fluctuating trophic pressures. In contrast with upper jaw length, selection has increased gill raker number in the open-water population. Differences in gill raker number between ecotypes are asso-

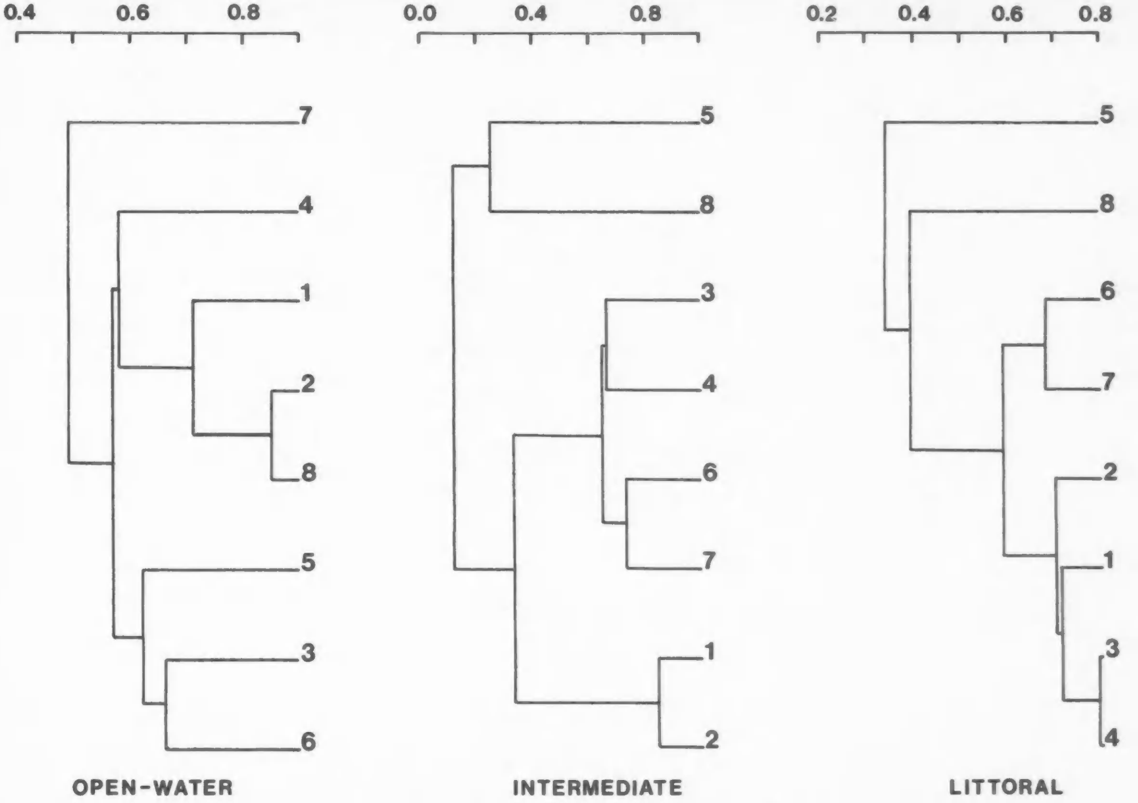

FIG. 3. Trophic character organization based on cluster analysis of genetic correlations among traits, within ecotype. 1 = head length; 2 = snout length; 3 = eye diameter; 4 = upper jaw length; 5 = gill raker number; 6 = head depth; 7 = inner orbital width; 8 = gill raker length.

TABLE 4. Selection gradients for transitions between population trophic morphologies. The genetic covariance matrices from the intermediate and littoral morphs were used to compute the pooled within-populations genetic covariance matrix *G*.

	Morphotype transition		
	Intermediate/ open-water	Intermediate/ littoral	Open-water/ littoral
HEAL	−0.005	0.416	0.422
SNOL	0.790	−0.131	−0.921
EYED	−0.992	−0.275	0.716
UPJL	1.246	0.055	−1.191
GRL	0.262	0.141	−0.221
HEAD	−1.145	−0.098	1.046
INOW	0.516	0.268	−0.247
GRN	−1.064	0.535	1.117

ciated with differences in foraging efficiency on water-column prey (Lavin and McPhail 1986). The open-water population is a superior open-water forager compared with the littoral in laboratory experiments.

The morphology of the intermediate ecotype provides a benthic foraging efficiency like that of the littoral population, but a water-column foraging ability like that of the open-water population (Lavin and McPhail 1986). The patterns of genetic correlations between upper jaw length and gill raker number facilitate this response to an intermediate trophic regime. Weak genetic correlations between gill raker architecture and head shape traits allow selection to act independently on elements of each (i.e. upper jaw length and gill raker number). Less selection is required to shift phenotypic means when traits are weakly correlated than if traits are either strongly or negatively correlated (Lande 1979); this allows a more rapid and direct approach to a phenotypic optimum. Strong genetic correlations between gill raker number and head shape characters would result in a suboptimal approach to the best adapted phenotype ("genetic slippage"; Dickerson 1955), and the population as a whole would incur a greater selective load.

The adaptive divergence of morphology along a gradient, or axis, of particle size has been recognized previously in interspecific comparisons of Darwin's ground finches (*Geospisa*; Schluter and Grant 1984) and anoles (Lister 1976); it is of particular interest to the evolution of *Gasterosteus* for two reasons.

The biological species pairs of *Gasterosteus aculeatus* which occur sympatrically in Enos Lake on Vancouver Island (McPhail 1984) are strongly divergent on diet type, which can be characterized by differences in particle size (Bentzen 1982). The female of the "limnetic" species is an obligate planktivore and has characters exaggerated beyond those of our open-water ecotype for plankton foraging (Bentzen and McPhail 1984).

Hence, there is certainly potential for segregation along a trophic axis, contributing to the origin of these species pairs. Ratcliffe and Grant (1983) have shown that reproductive isolation can be based on the recognition of interspecific differences in trophic morphology.

The response of gill raker number and upper jaw length to variable trophic environments appears general for lacustrine populations of *Gasterosteus aculeatus* in British Columbia. Populations inhabiting large lakes dominated by pelagic regions are characterized by numerous gill rakers (Hagen and Gilbertson 1972; Lavin and McPhail 1986) and short jaws (Lavin and McPhail 1986), relative to populations from shallow lakes dominated by benthic production. We therefore suggest that variation in diet type is a common selective force organizing part of the interpopulation variability characteristic of the *Gasterosteus aculeatus* species complex.

Clearly, there are other axes of adaptive divergence which contribute to interpopulation mophological variability (Hagen 1967; Hagen and Gilbertson 1973). We have addressed only one of an infinite number of potential selective axes, although it is one frequently correlated with the radiation of teleost morphology (Lindsey 1981; review in Greenwood 1984). In addition, we have not examined all possible trophic variables. There may be selective forces acting on a host of unmeasured characters; however, as always, a partial resolution of the problem is better than none at all (Lande and Arnold 1983; Schluter 1984).

Interpopulation patterns of genetic correlation suggest that the within-populations covariance matrix has remained approximately constant during the 10 500 yr of evolution within this system. The few studies which have investigated interpopulation differences in *G* indicate that this apparent stability is a general phenomenon (Arnold 1981; Lofsvold 1986; Mitchell-Olds 1986). However, interspecific comparisons of *G* have shown reorganization of *G* (Lofsvold 1986). It is unknown how much selection is required to alter genetic covariance, or what effects founder episodes (Carson and Templeton 1984) can produce. Laboratory investigations have shown patterns of genetic correlation to be preserved under directional selection (Cheung and Parker 1974; Leamy and Atchley 1984). But Lande (1980) demonstrated that covariance should evolve given small effective population size or strong selection.

As yet, the measurement of genetic covariance is one of few techniques for quantifying genetic architecture, albeit pleiotropic and linkage arrangements only. The stability of *G* which we observe indicates that lacustrine populations of *Gasterosteus aculeatus* in the upper Cowichan drainage have responded to contrasting selective regimes with extant genetic architecture rather than genomic reorganization. Hence, the character organization was sufficient for adaptive divergence of trophic morphology in this system. Conversely, one might consider the morphological response of these populations to be constrained by *G*. A more loosely integrated set of trophic characters would permit a much greater array of morphological solutions to similar adaptive problems.

The quantification of genetic covariance is crucial to understanding morphological divergence because additive genetic parameters measure epigenetic constraints on the direction and rate of evolution (Cheverud 1984). With no sense of the way in which characters are organized to respond to selection, we cannot hope to assess biological adaptation and hence elucidate processes of morphological evolution. However, the investigation of adaptive divergence also requires a knowledge of both the organism's ecology (Clarke 1978) and the functional significance of a given trait(s) (Bock 1980). The preceding studies in this investigation have demonstrated (1) nonrandom association of trophic morphology with habitat and diet (Lavin and McPhail 1985) and (2) functional significance of ecotypic divergence in upper jaw length and gill raker morphology (Lavin and McPhail 1986). Here, we have sought to identify those characters which have been selectively modified and to describe the organization of characters in order to understand that response. We feel that this combined approach addresses the demands in the literature for more precise explanations of phenotypic variability (e.g. Lewontin 1978; Gould and Lewontin 1979).

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Genetic Identification of Cutthroat Trout, *Salmo clarki*, in Glacier National Park, Montana

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Trout populations in 29 lakes in Glacier National Park were identified by meristic and electrophoretic analyses to assess the extent of introgressive hybridization between introduced nonnative trout and the indigenous cutthroat trout, *Salmo clarki lewisi*. Native cutthroat trout remain in 16 lakes draining to the North and Middle forks of the Flathead River; no native trout were found east of the Continental Divide. Introduced Yellowstone cutthroat trout, *Salmo clarki bouvieri*, occur in six headwater lakes. Hybrid populations, including both *S. c. lewisi* × *bouvieri* and *S. clarki* × *S. gairdneri*, inhabit six lakes. Hybridization between native and introduced trouts has been minimal, apparently due to strong selective pressures favoring the indigenous genotype. Close agreement was observed between the meristic and electrophoretic results.

Des populations de truites de 29 lacs du parc national Glacier ont été identifiées par analyses méristiques et électrophorétiques dans le but d'évaluer l'importance de l'hybridation introgressive entre les truites non indigènes introduites et les truites fardées indigénes (*Salmo clarki lewisi*). Les truites fardées indigènes occupent toujours 16 lacs se déversant dans les fourches North et Middle de la rivière Flathead. Aucune truite indigène n'a été décelée à l'est de la ligne de partage des eaux continentales. Des truites fardées de Yellowstone (*Salmo clarki bouvieri*) introduites occupaient six lacs d'amont. Des populations hybrides de *S. c. lewisi* × *bouvieri* et de *S. clarki* × *S. gairdneri* ont été décelées dans six lacs. Il y a eu très peu d'hybridation entre les poissons indigènes et introduits. Il semble que ce phénomène soit dû à de fortes pressions sélectives en faveur du génotype indigène. Il y avait concordance étroite entre les résultats des analyses méristiques et électrophorétiques.

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Taxonomy of the native trout of Glacier National Park has existed in a state of confusion until fairly recently. This can be traced to the belief of Jordan and Evermann (1896) that a single subspecies of cutthroat trout, *Salmo clarki clarki*, ranged from the coastal waters of the Pacific Northwest inland to the headwaters of the Columbia River system. Cutthroat trout east of the Continental Divide, including those native to the South Saskatchewan and Upper Missouri River drainages and the Yellowstone Lake basin, were classified as *S. clarki lewisi*. This scheme recognized both subspecies as part of Glacier Park's native ichthyofauna (Schultz 1941). It is now known that the coastal cutthroat trout, *S. c. clarki*, extended inland only as far as the Cascade Range and, consequently, never reached the Glacier Park area.

East of the Cascades, the "westslope" cutthroat trout, *S. c. lewisi*, historically ranged from west-central Washington across Idaho, and into western Montana, where it crossed the Continental Divide and entered portions of the South Saskatchewan and Upper Missouri River basins. Behnke (1979) attributed this distribution to headwaters crossover in the vicinity of Glacier Park. Along the western periphery of this range (e.g. St. Joe, Clark Fork, and Kootenay rivers), *S. c. lewisi* has been isolated from rainbow trout, *S. gairdneri*, by upstream barriers.

Studies of protein variation (Loudenslager and Thorgaard 1979; Loudenslager and Gall 1980) and morphomeristic character analyses (Roscoe 1974; Behnke 1979) have indicated that trout indigenous to the Snake River above Shoshone Falls and throughout the Yellowstone River drainage are distinct from *S. c. lewisi* and represent a markedly different evolutionary lineage. The name *S. clarki bouvieri* has been assigned to the Yellowstone cutthroat trout (Behnke 1979). *Salmo clarki lewisi* is, therefore, the only subspecies of cutthroat trout indigenous to Glacier Park. Although historically present in all three continental drainages that head in the park, populations in the Missouri and Saskatchewan River drainages appear to have been restricted to downstream reaches outside the existing park boundary.

A fundamental tenet of the National Park Service's Organic Act of 1916 is preservation of the indigenous biota of the national parks. However, early acceptance of sport fishing as a legitimate use of park resources created a management dilemma, particularly regarding the issue of artificial enhancement of fishery resources. Lack of a comprehensive fisheries policy prior to World War II allowed the momentum of established fish stocking practices to prevail over the Service's otherwise straightforward philosophy of nonmanipulative ecosystems management. In recent decades, attitudes have shifted back toward maintenance of self-sustaining indigenous fisheries in the Natural Area category of national parks.

Between 1912 and the post-World War II years, tens of millions of nonnative salmonid eggs, fry, and fingerlings were stocked in Glacier Park waters. The Yellowstone cutthroat trout was the principal species stocked; however, other species included the rainbow trout; brook trout, *Salvelinus fontinalis*; lake trout, *Salvelinus namaycush;* lake whitefish, *Coregonus clupeaformis*; and kokanee, *Oncorhynchus nerka*. Lake trout are native to a few park waters in the South Saskatchewan drainage, but have been introduced into several large lakes west of the Divide. Stocking diminished in Glacier Park in the 1950's and was terminated in the early 1970's.

Prior to this work, the impact of 60 yr of fish stocking on the native trout was unknown. We undertook this study to document (1) the extent of hybridization between introduced salmonids and the indigenous cutthroat trout and (2) the present distribution of native, introduced, and hybrid trout populations within the boundaries of Glacier Park.

Study Area

Glacier National Park adjoins the U.S.–Canada border in the northwest corner of Montana and encompasses approximately 410 000 ha of precipitous mountain terrain and glacial valleys. Approximately 650 lakes larger than 2 ha and 2600 km of streams drain from 2500 m MSL (elevation above mean sea level) to approximately 1000 m elevation. Lower and mideelevation forests are principally composed of lodgepole pine, *Pinus contorta*. Tundra-like conditions prevail above 2400 m MSL.

The headwaters of three continental drainages originate in Glacier Park. Waters west of the Continental Divide enter the North and Middle forks of the Flathead River and drain to the Pacific Ocean via the Columbia River. East of the mountains, a second divide separates the South Saskatchewan (Hudson Bay) drainage from the headwaters of the Upper Missouri Basin. Fisheries occur in at least 64 park lakes; 47 waters known or suspected to contain resident cutthroat trout were surveyed (Fig. 1). Lakes were selected for surveying on the basis of stocking records, creel census data, and recommendations by backcountry rangers.

Materials and Methods

Cutthroat trout were collected by gill nets in the lakes and by electroshocking in streams. A numbered tag was affixed to each specimen for subsequent comparison of meristic and electrophoretic data among individual trout. The right eye, a section of liver, and 1 cm^3 of muscle tissue were removed, placed in coded plastic bags, and frozen on-site for later analyses by starch–gel electrophoresis. Processed trout were preserved in 10% formalin and transported to the laboratory.

Morphomeristic and electrophoretic analyses were performed independently on the same specimens. Genetic identifications were subsequently based on a consideration of the results from both procedures.

Gillrakers were counted on the anterior and posterior sides of the first left gill arch. Basibranchial teeth were stained with alizarin-red dye and counted under a binocular microscope. scales in the lateral series (counted two rows above the lateral line) and pyloric caeca were also counted. Meristic data were compared with typical values cited for *S. c. lewisi* and *S. c. bouvieri* by Roscoe (1974) and Behnke (1979; and unpubl. data) (Table 1). Close attention was also paid to the shape, relative size, and distribution of spotting.

The protein products of 42 loci encoding 19 enzymes (Leary et al. 1984) were analyzed in the specimens. Sample preparation and electrophoresis in starch gels followed the methods of Utter et al. (1974) with the stains and buffer systems of Allendorf et al. (1977). Isozyme loci are designated with the nomenclature described by Allendorf et al. (1983).

Results and Discussion

Forty-seven waters comprising 9462 ha were surveyed during the summers from 1978 through 1985. Twenty-nine lakes encompassing 1487 ha contained cutthroat trout or hybrid trout populations; 818 specimens were collected for examination. Fourteen lakes known to have been stocked with cutthroat trout no longer contain fish or harbor species other than cutthroat trout. These include Long Bow, Pocket, Numa, and Dutch lakes (North Fork, Flathead River drainage); Flattop and Ellen Wilson lakes (Middle Fork, Flathead River drainage); and Stoney Indian, Poia, Kootenai, Cosely, Glenns, Waterton, and St. Mary lakes, and the Belly River (South Saskatchewan River drainage) (Fig. 1).

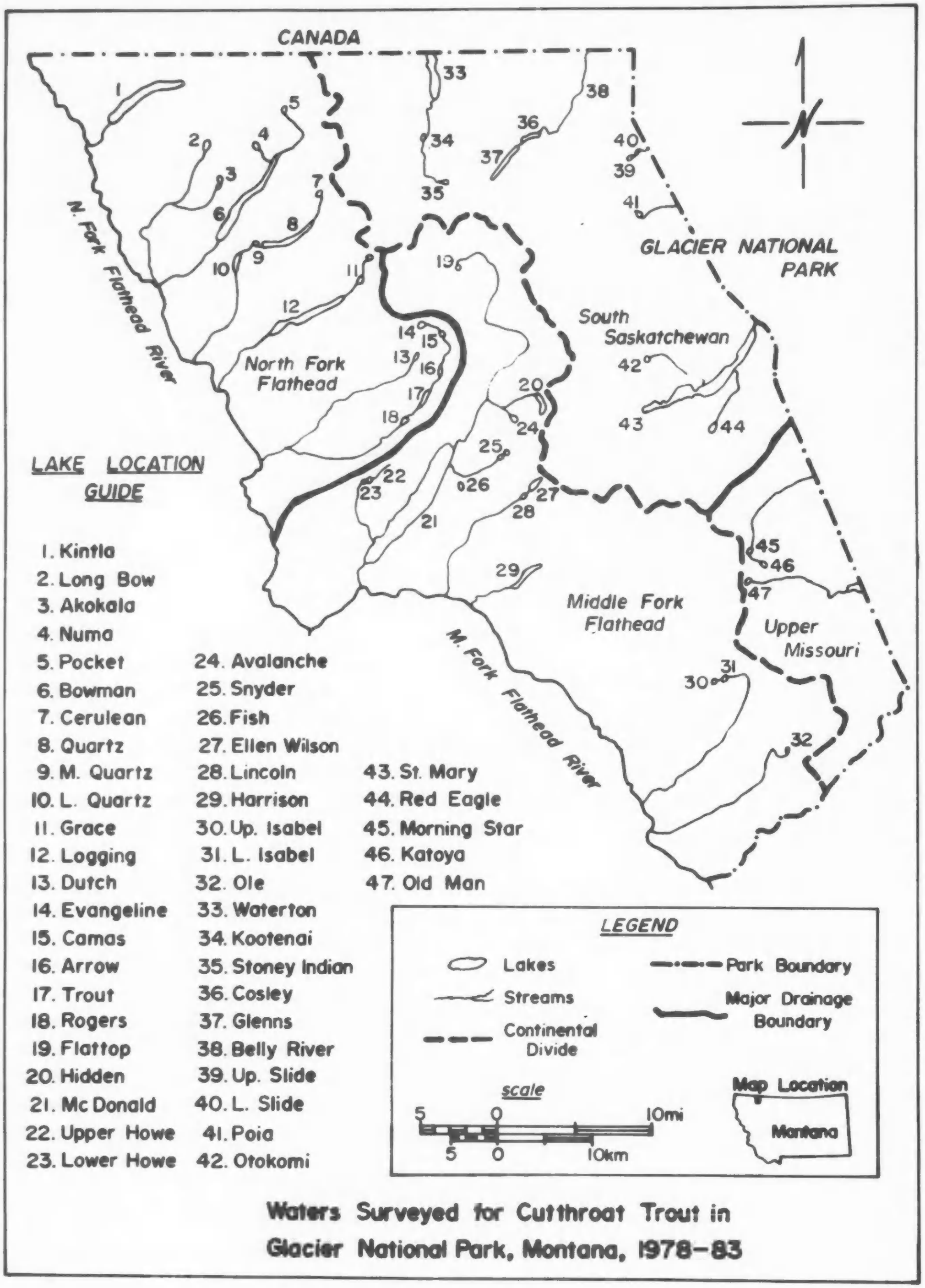
Data for McDonald, Bowman, and Kintla lakes were discarded because ecological disturbances caused by nonnative fish introductions have impacted cutthroat trout populations in these waters to the point where adequate samples could not be obtained. Also, only six specimens were collected from Rogers Lake, a 34-ha bog in an advanced stage of succession. Results for this water were similarly excluded, since the lake probably does not harbor a resident trout population.

Pure strain populations of *S. c. lewisi* were found in seven lakes draining to the North Fork, Flathead River, and in nine lakes in the Middle Fork, Flathead River. Due to close proximity of several of the lakes, the 16 lacustrine populations of *S. c. lewisi* are organized into 14 geographically distinct groups (Table 2). No populations of *S. c. lewisi* were found east of the Continental Divide.

Three North and Middle Fork lakes and three waters in the Upper Missouri drainage contained exclusively Yellowstone cutthroat trout. Six additional lakes harbored various combinations of hybrids (Table 3). Yellowstone cutthroat trout and hybrid populations occurred, with one exception, in lakes that were previously barren of fish. Typical native and Yellowstone cutthroat trout phenotypes are illustrated in Fig. 2.

Meristics

Meristic characters found most useful for separation of the two types of cutthroat trout were (1) anterior gill rakers, (2) posterior gill rakers, and (3) basibranchial teeth. The mean number of basibranchial teeth, when plotted against the mean number of posterior gill rakers, readily separated *S. c. lewisi* from *S. c. bouvieri* (Fig. 3).


CANADA
GLACIER NATIONAL PARK
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N. Fork Flathead River
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South Saskatchewan
Middle Fork Flathead
Upper Missouri
M. Fork Flathead River
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LAKE LOCATION GUIDE
1. Kintla
2. Long Bow
3. Akokala
4. Numa
5. Pocket
6. Bowman
7. Cerulean
8. Quartz
9. M. Quartz
10. L. Quartz
11. Grace
12. Logging
13. Dutch
14. Evangeline
15. Camas
16. Arrow
17. Trout
18. Rogers
19. Flattop
20. Hidden
21. Mc Donald
22. Upper Howe
23. Lower Howe
24. Avalanche
25. Snyder
26. Fish
27. Ellen Wilson
28. Lincoln
29. Harrison
30. Up. Isabel
31. L. Isabel
32. Ole
33. Waterton
34. Kootenai
35. Stoney Indian
36. Cosley
37. Glenns
38. Belly River
39. Up. Slide
40. L. Slide
41. Poia
42. Otokomi
43. St. Mary
44. Red Eagle
45. Morning Star
46. Katoya
47. Old Man
LEGEND
Lakes
Streams
Continental Divide
Park Boundary
Major Drainage Boundary
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Map Location
Montana
Waters Surveyed for Cutthroat Trout in Glacier National Park, Montana, 1978-83

FIG. 1. Waters surveyed in Glacier National Park, Montana.

TABLE 1. Ranges of meristic character values for *S. c. lewisi*, *S. c. bouvieri*, and *S. gairdneri* used for identification of specimens from Glacier National Park, Montana. Typical mean values are given in parentheses. (Adapted from Roscoe 1974; Behnke 1979)

Character	*S. c. lewisi*	*S. c. bouvieri*	*S. gairdneri*
Gillrakers			
Anterior	16–21 (17–19)	18–24 (20–21)	16–21 (18–20)
Posterior	0–5 (1–3)	5–15 (7–10)	0
Basibranchial teeth	1–15 (4–8)	6–40 (22)	0

TABLE 2. Lakes in Glacier National Park containing native cutthroat trout, *S. c. lewisi*.

Name of water	Area (ha)	Origin of population
North Fork, Flathead River drainage		
Akokala L.	9	Indigenous
Cerulean L.	20	Indigenous
Quartz L.	349	Indigenous
Middle Quartz L.	19	Indigenous
Lower Quartz L.	67	Indigenous
Logging L.	444	Indigenous
Trout L.	86	Indigenous
Middle Fork, Flathead River drainage		
Avalanche L.	23	Introduced
Snyder L.	2	Introduced
Upper Howe L.	3	Introduced
Lower Howe L.	12	Introduced
Lincoln L.	14	Indigenous
Harrison L.	101	Indigenous
Upper Isabel L.	6	Indigenous
Lower Isabel L.	17	Indigenous
Ole L.	2	Indigenous

TABLE 3. Electrophoretic classification of nonnative and hybrid trout populations in Glacier National Park.

Name of water	Area (ha)	Type of trout[a]	Sample size	
North Fork, Flathead River				
Grace L.	32	YCT × WCT	30	
Evangeline L.	28	YCT	30	
Camas L.	8	YCT	30	
Arrow L.	23	WCT × YCT	30	
Middle Fork, Flathead River				
Hidden L.	110	YCT	30	
Fish L.	3	WCT × YCT	31	
South Saskatchewan drainage				
Otokomi L.	9	YCT × RT	23	
Upper Slide L.[b]	5	YCT × RT	12	30
Lower Slide L.	15	YCT × RT	18	
Upper Missouri drainage				
Katoya L.	4	YCT	30	
Old Man L.	17	YCT	30	
Morning Star L.	4	YCT	11	

[a] RT = *S. gairdneri*; WCT = *S. clarki lewisi*; YCT = *S. c. bouvieri*. Symbol of the dominant phenotype appears first in the case of hybrids.

[b] Upper and Lower Slide lakes contain the same trout population.

The meristic index represents the sum of the mean values of the three principal characters (Table 4). Usefulness of this index derives from the fact that the mean value of all three characters is higher for *S. c. bouvieri* than for *S. c. lewisi*. Thus, small differences in mean counts of individual characters within any given sample are amplified. This more clearly separates the two subspecies of cutthroat trout.

Index values for cutthroat hybrids tended to be intermediate; however, some overlap occurred between the upper values for hybrids of *S. c. lewisi* × *bouvieri* and the lower values for *S. c. bouvieri*. Thus, the meristic index is best suited for separating genetically pure populations of the two cutthroat trout subspecies. Hybridization between these forms was indicated by atypical pigmentation. Native trout typically displayed small irregularly shaped spots with very few (i.e. less than 2% of total) located below the lateral line anterior to the anal fin. The Yellowstone subspecies by contrast exhibits larger spots, essentially round in shape, with 10–15% of them lying anterior to the anal fin below the lateral line (see Fig. 2). Hybrids of *S. c. lewisi* × *bouvieri* usually revealed some deviation from the typical shape, size, and/or distribution of lateral spotting.

Hybrid influence from rainbow trout into either *S. c. lewisi* or *S. c. bouvieri* was inferred from anomalous spotting on the snout and any notable increase in the density of lateral spotting accompanied by a decrease in the relative size of spots. Examination of stocking records confirmed exposure to *S. gairdneri* in all samples where rainbow trout influence was suspected on the basis of atypical spotting patterns. Tentative identification of rainbow trout influence in cutthroat trout was consistently confirmed by the electrophoretic results.

Electrophoresis

Previous examination (Leary et al. 1984) revealed that 8 of 42 enzyme loci examined could be used to differentiate among the two cutthroat trout subspecies and rainbow trout (Table 5). Electrophoretic classifications of nonnative and hybrid trout populations are presented in Table 3. Populations listed in Table 2 were determined by electrophoresis to be *S. c. lewisi*. Slight discrepancies in sample sizes (*N*) for the meristic and electrophoretic analyses occurred because a few specimens were mutilated by the gill nets to such an extent that meristic characters could not be accurately counted and because accidental thawing destroyed a few electrophoretic samples.

Native Cutthroat Trout

Although *S. c. lewisi* is indigenous to all three continental drainages with headwaters in Glacier Park (Hanzel 1959; Roscoe 1974; Behnke 1979), the subspecies was evidently unable to gain access to the headwater lakes of the South

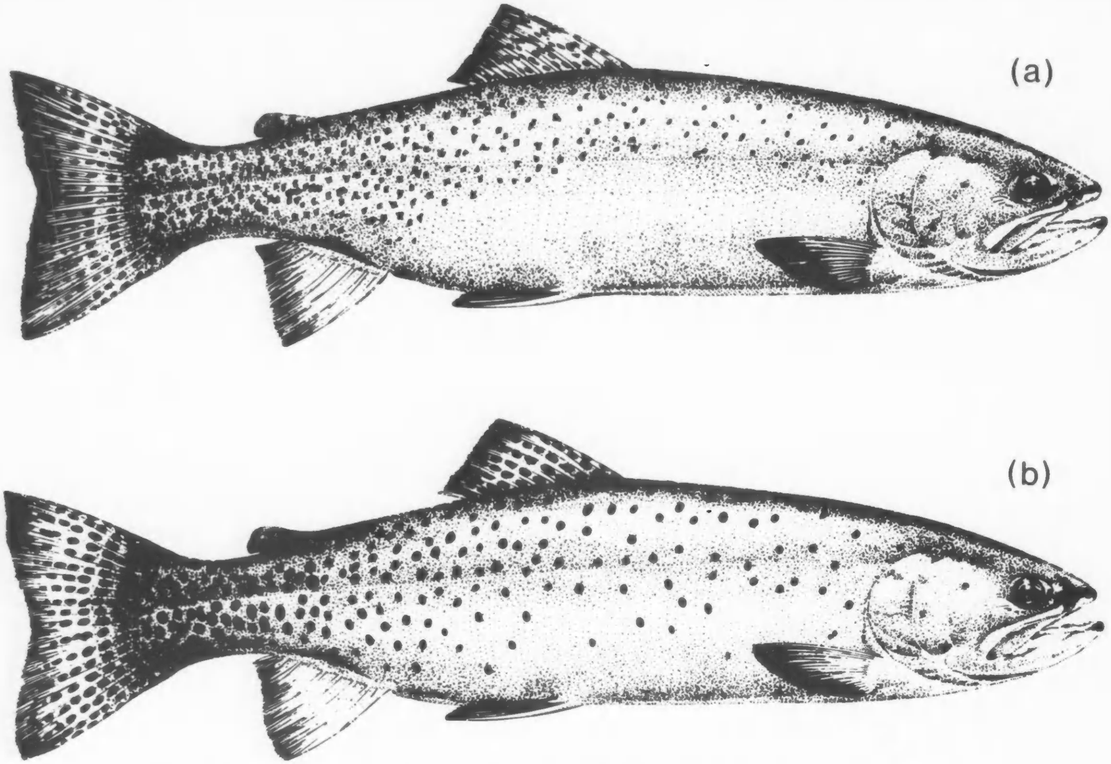

FIG. 2. Illustrations of (a) westslope (*S. c. lewisi*) and (b) Yellowstone (*S. c. bouvieri*) cutthroat trout.

Saskatchewan and Missouri River drainages. Thus, trout found east of the Divide in Glacier Park today are nonnative species, subspecies, or hybrids whose origins can be traced directly to introductions dating from about 1910.

Surviving native trout populations in Glacier Park reside exclusively in waters of the North and Middle Fork drainages of the Flathead River system, including 12 lakes (1134 ha) where the subspecies is believed indigenous (Table 2). Native trout also occur in four small lakes (about 40 ha) that drain to McDonald Lake; included are Upper and Lower Howe, Snyder, and Avalanche lakes. We believe, however, that these populations were introduced, the parent stocks probably originating from McDonald Lake. All four lakes are drained by high-gradient streams containing numerous barriers.

Yellowstone Cutthroat Trout

Introduced Yellowstone cutthroat trout inhabit six park lakes (Table 3). It is noteworthy that *S. c. bouvieri* has not replaced or significantly hybridized with native trout in any water where an indigenous population was present. The Yellowstone subspecies has been able to sustain itself in Glacier Park only in small, high-elevation lakes previously barren of fish.

Hybrid Cutthroat Trout

Given the scale of fish stocking that occurred throughout Glacier Park during the first half of the century, the incidence of hybridization between native and introduced cutthroat subspecies has been minimal. Moreover, no single circumstance appears responsible. Genetic mixing of native and introduced trout has apparently resulted from circumstances unique to specific waters.

Arrow Lake

Of the three park lakes containing hybrid cutthroat trout populations, only Arrow Lake (North Fork) was inhabited by native trout prior to the introduction of *S. c. bouvieri*. The meristic index for the sample was 24.1, typical of *S. c. lewisi*, but five specimens contained no basibranchial teeth, which depressed the mean value for that character. Two specimens captured in the outlet stream possessed 13 and 15 basibranchial teeth, respectively, typical for *S. c. bouvieri*. Several specimens also displayed subtle indications of hybrid influence from Yellowstone cutthroat trout in the form of larger, more evenly distributed lateral spots.

Enzyme analysis confirmed hybridization between westslope and Yellowstone cutthroat trout in this lake; 88% of the alleles at six diagnostic loci were of westslope cutthroat trout origin (Table 6). Twenty-three of the 30 fish sampled were homozygous for the westslope allele at all six loci; five fish were heterozygous for both westslope and Yellowstone alleles at one or more loci, and the two specimens taken from the outlet were homozygous for the Yellowstone allele at all diagnostic loci. There is a significant deficiency of heterozygotes at all six diagnostic loci in this sample compared with expected (i.e. Hardy–Weinberg) proportions. Thus, this sample does not

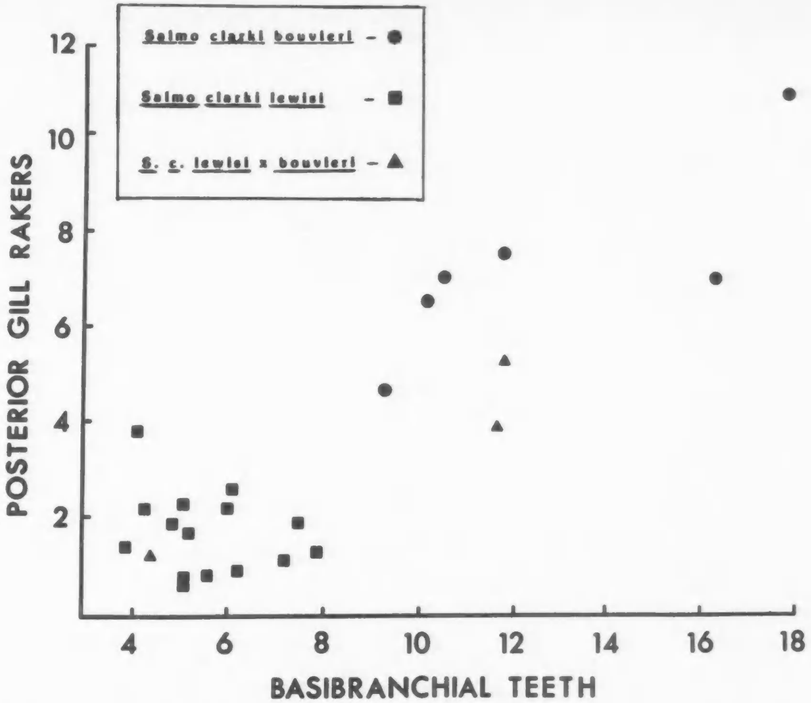

FIG. 3. Separation of *S. c. lewisi* and *S. c. bouvieri* samples by plot of mean number of posterior gill rakers against mean number of basibranchial teeth.

represent a random-mating hybrid swarm. The population is predominately westslope cutthroat trout exhibiting slight hybrid influence from the Yellowstone subspecies.

The stocking of Arrow Lake with 112 000 eggs and 9720 fry of *S. c. bouvieri* between 1924 and 1935 could account for the observed hybrid influence. However, a more plausible explanation is that Yellowstone cutthroat trout introduced upstream in Evangeline and Camas lakes periodically descend over a 5-m falls and enter Arrow Lake where they spawn. This interpretation is based on the low level of hybridization detected in the population and by the presence of two juvenile specimens of apparently pure *S . c. bouvieri* collected in Camas Creek below Arrow Lake; these fish could only have come from Camas or Evangeline lakes (see Fig. 1).

Fish Lake

There is no record of trout introductions into Fish Lake, but National Park Service file correspondence from the 1920's mentions stocking of this water. Although Schultz (1941) identified the population as native cutthroat trout, our data indicate that it is a hybrid swarm of *S. c. lewisi* × *bouvieri*, the most extreme example of this condition found. The meristic index for the sample was 35.9 and a wide range of coloration and spotting patterns was displayed, including phenotypes typical of both subspecies; many specimens exhibited intermediate spotting characteristics. Twenty-nine of the 31 trout examined contained electrophoretic alleles from both *S. c. lewisi* and *S. c. bouvieri*. All three electrophoretic phenotypes were detected at all marker loci, suggesting random mating within the population. Two specimens possessed only *S. c. bouvieri* alleles at the marker loci, but were probably hybrids that happened to possess exclusively Yellowstone alleles at the diagnostic loci by chance alone.

Fish Lake does not have an inlet or outlet stream suitable for trout spawning, although during high water it discharges across a bog to nearby Crystal Creek. It is possible that the lake may contain areas of suitable substrate and oxygen to permit trout spawning and egg incubation. Successful lake spawning is known for cutthroat trout and rainbow × cutthroat hybrids in several remote lakes in Rocky Mountain National Park, Colorado (Rosenlund 1980), and similar occurrences have been noted for cutthroat trout in Bigelow and Hay lakes in western Montana (J. Huston, Montana Department of Fish, Wildlife, and Parks, 490 No. Meridian Ave., Kalispell, MT 59901, pers. comm.). Penlington (1983) also reported spawning of rainbow trout in Rotoma Lake, New Zealand.

In the absence of any competitive advantage conferred upon either subspecies by a long period of prior residence, and with minimal opportunity for escapement, random mating between *S. c. lewisi* and *S. c. bouvieri* best explains the observed hybrid swarm in Fish Lake. Similar hybrid swarms involving these subspecies have been described by Gyllensten et al. (1985).

Grace Lake

Grace Lake covers approximately 32 ha and is located above a 15-m waterfall in the headwaters of the Logging Creek drainage (North Fork, Flathead River). The historically fishless water was stocked with 101 000 cutthroat trout eggs in 1925. Analysis of 30 specimens indicates a hybrid population of *S. c. bouvieri* × *lewisi* possessing predominately Yellowstone cutthroat characteristics. We believe that the 1925 plant contained a mix of eggs from both cutthroat types that gave rise to a hybrid

TABLE 4. Mean values of principal meristic characters for cutthroat trout collected from Glacier National Park. Standard deviation values are given in parentheses.

Population	*N*	Gill rakers		Basibranchial teeth	Meristic index
		Anterior	Posterior		
		North Fork, Flathead River drainage			
Akokala L.	31	19.6 (±0.9)	1.8 (±1.8)	7.6 (±2.7)	29.0
Cerulean L.	17	18.6 (±1.4)	0.6 (±0.6)	5.1 (±3.5)	24.3
Quartz L.	30	18.1 (±0.9)	0.9 (±0.6)	6.2 (±3.8)	25.2
Middle Quartz L.	30	18.8 (±0.8)	0.7 (±0.7)	5.1 (±2.8)	24.6
Lower Quartz L.	31	18.0 (±1.2)	2.2 (±1.6)	6.0 (±3.9)	26.2
Grace L.	23	21.2 (±1.2)	5.3 (±2.9)	11.9 (±6.3)	38.4
Logging L.	35	19.0 (±1.2)	1.1 (±0.8)	7.4 (±4.2)	27.5
Evangeline L.	30	20.7 (±1.1)	11.0 (±2.8)	18.5 (±6.0)	50.2
Camas L.	15	20.3 (±1.2)	7.3 (±3.3)	10.5 (±6.1)	38.1
Arrow L.	28	18.4 (±0.9)	1.2 (±0.6)	4.5 (±3.5)	24.1
Trout L.	30	17.8 (±1.0)	2.6 (±1.3)	6.1 (±3.7)	26.5
		Middle Fork, Flathead River drainage			
Hidden L.	17	19.9 (±1.4)	7.6 (±4.1)	11.4 (±4.2)	38.9
Avalanche L.	29	19.0 (±1.2)	1.3 (±0.9)	7.9 (±4.7)	28.2
Snyder L.	33	18.3 (±1.1)	2.1 (±1.1)	4.3 (±2.2)	24.7
Fish L.	29	20.3 (±1.3)	3.8 (±2.8)	11.6 (±6.2)	35.7
Upper/Lower Howe L.	24	18.3 (±1.2)	1.7 (±1.5)	4.8 (±3.4)	24.8
Lincoln L.	16	17.4 (±1.9)	3.8 (±1.7)	4.1 (±2.7)	25.3
Harrison L.	30	17.3 (±0.8)	0.8 (±0.8)	5.6 (±3.0)	23.7
Upper Isabel L.	8	19.0 (±0.8)	2.6 (±0.5)	5.1 (±4.2)	26.7
Lower Isabel L.	30	18.5 (±0.8)	1.4 (±1.3)	3.9 (±5.1)	23.8
Ole L.	30	18.9 (±1.0)	1.9 (±1.3)	5.1 (±3.3)	25.9
		Upper Missouri River drainage			
Katoya L.	30	20.5 (±1.0)	7.1 (±3.0)	16.4 (±5.6)	44.0
Old Man L.	30	20.5 (±1.3)	4.6 (±1.9)	8.6 (±6.2)	33.7
Morning Star L.	11	20.0 (±1.0)	6.6 (±2.2)	10.2 (±5.1)	36.8

TABLE 5. Loci differentiating westslope cutthroat trout, Yellowstone cutthroat trout, and rainbow trout. Values represent electrophoretic mobility relative to the standard common allele of rainbow trout.

Enzyme	Locus abbreviation	Relative allelic mobility		
		Rainbow trout	Westslope cutthroat	Yellowstone cutthroat
Creatine kinase	Ck-2	100	84	84
Glucophosphate isomerase	Gpi-3	100	92	100
Isocitrate dehydrogenase	Idh-1	100	100	−75
Isocitrate dehydrogenase	Idh-3,4	100, 40 71, 114	100, 86 71, 40	100 71
Malic enzyme	Me-1	100, 57	88	100
Malic enzyme	Me-4	100, 75	100	110
Sorbitol dehydrogenase	Sdh	40, 100 200	40	100

population. This hypothesis is supported by Schultz's (1941) observation of two distinct spotting patterns among trout in Grace Lake. Specimens collected during the present study displayed intermediate pigmentation and the meristic index was 38.4. The enzyme results from Grace Lake show the presence of both westslope and Yellowstone alleles. Approximately 25% of the genes at the Me-1, Me-4, and Gpi-3 loci were of westslope origin, while the remaining 75% were of Yellowstone origin.

TABLE 6. Electrophoretic analysis of hybrids between westslope and Yellowstone cutthroat trout sampled from Glacier National Park lakes. P is the proportion of genes that are of westslope cutthroat trout origin; F is the fixation index and is the proportional deficit of heterozygotes ($F = 1 - (H_o/H_e)$), where H_o is the observed proportion of heterozygotes and H_e is the expected proportion of heterozygotes. Dashes indicate no data available. All loci could not be scored in some samples because of denaturation of some liver enzymes.

	Fish Lake		Arrow Lake		Grace Lake	
Locus	P	F	P	F[a]	P	F
Gpi-3	0.290	0.076	0.900	0.636***	0.348	0.277
Idh-1	0.387	0.064	0.833	0.523**	0	—
Idh-3,4	0.242	−0.298	0.900	0.636***	—	—
Me-1	0.258	−0.160	0.900	1.000***	0.100	0.272
Me-4	0.306	0.174	0.850	0.614***	0.317	0.167
Sdh	—	—	0.900	0.636***	—	—
Mean	0.297	−0.028	0.881	0.674***	0.191	0.239

[a] Test for departure from expected genotypic distribution with $F = 0$; $^{**}P < 0.01$; $^{***}P < 0.001$.

There is no evidence of nonrandom mating based on phenotypes at these three loci: however, all 30 specimens were homozygous for the Yellowstone allele at Idh-1. It is unlikely that such a large difference between loci in the proportion of westslope and Yellowstone alleles could be due to chance alone. There is less than one chance in a million of sampling only Yellowstone alleles in 30 fish if the frequency of the westslope allele is 0.25. Evidence from similar hybrid swarms of these subspecies examined by Gyllensten et al. (1985) suggest that such heterogeneity may result from genetic drift.

Cutthroat × Rainbow Trout Hybrids

Three headwater lakes in the South Saskatchewan drainage contained Yellowstone × rainbow trout hybrids; Otokomi, Upper Slide, and Lower Slide, collectively encompassing 29 ha (Table 3). These lakes were stocked with cutthroat trout during the 1930's. Undocumented introductions of rainbow trout may have occurred, but it is also possible that Yellowstone cutthroats placed in these waters were mixed with rainbow trout, perhaps inadvertently at the hatchery. The Otokomi and Slide Lake populations were predominately Yellowstone cutthroat trout possessing small and variable amounts of rainbow trout characteristics, as evidenced by spotting patterns and the presence of electrophoretic alleles from rainbow trout at Ck-2, Idh-1, Idh-3, and Me-4.

Although the sample size for Red Eagle Lake was too small to allow definitive conclusions, the specimens collected appeared to represent rainbow trout possessing some hybrid influence from Yellowstone cutthroat trout. Reduced basibranchial teeth counts, anomalous spotting patterns, and electrophoresis all indicated slight contamination from *S. c. bouvieri*.

Competition

During the first half of the century, most park waters containing indigenous trout received multiple stockings of Yellowstone cutthroat trout. In view of the resulting low incidence of hybridization, it is evident that some combination of circumstances has strongly favored the indigenous subspecies.

The Yellowstone cutthroat trout evolved in a basin in which piscivorous fish species were absent. Having no evolutionary association with predatory fishes, *S. c. bouvieri* probably lacks the innate avoidance behavior necessary for coexistence. The native trout of Glacier Park, however, evolved with bull trout, *Salvelinus confluentus*, and the northern squawfish, *Ptychocheilus oregonensis*, both highly predaceous species. Therefore, *S. c. lewisi*, could be expected to possess a highly developed instinct to avoid piscivores.

Mortality among fry and juvenile Yellowstone cutthroat trout may have been especially high in Glacier Park waters immediately after planting. Native trout typically rear in the natal stream for an extended period, perhaps 2 or more yr, prior to entering a lake where they spend most of their adult life (Graham et al. 1980; Huston et al. 1984). Yellowstone cutthroat fry, however, characteristically migrate into lacustrine habitat within a few days after emergence and are believed to rear at depths below 20 m (Varley 1979). This probably has significant adaptive value for *S. c. bouvieri* in the Yellowstone ecosystem where predaceous fishes are absent. In Glacier Park waters, however, any tendency for *S. c. bouvieri* introduced as fry or fingerlings to gravitate toward moderate lake depths for rearing would expose them to significant predation. The situation may have been compounded by the concurrent invasion of lake trout into several park lakes. While this would also have affected native cutthroat populations, introduced *S. c. bouvieri* may have been severely impacted.

Another factor that may have contributed to the demise of Yellowstone cutthroat trout stocked in Glacier Park waters was the presence of the indigenous tapeworm, *Ceratocephalus* sp., prevalent in many native trout populations. The indigenous tapeworm differs from the native cestode of the Yellowstone basin, *Diphyllobothrium* sp., in that the life cycle of *Ceratocephalus* is confined exclusively to the aquatic environment where fish represent the final host; an as yet unidentified crustacean serves as intermediate host. *Diphyllobothrium*, however, is transmitted via mammal and avian hosts, mainly pelicans and seagulls, before passing to crustaceans and subsequently to fish, the latter serving as intermediate hosts (Varley and Schullery 1983).

It can be assumed that the two cutthroat subspecies, having evolved in the presence of different parasites, developed the means to coexist with the pathogens present in their respective habitats. Glacier Park's native cestode may have presented formidable problems for *S. c. bouvieri*. Animal hosts are often highly sensitive to parasites and pathogens which infect closely related host species (Barbehenn 1969). It is noteworthy that Yellowstone cutthroat trout have been successful in Glacier Park only in waters that were previously barren of fishes (and cestode parasites). Absence of *Ceratocephalus* from these lakes is probably due to the life cycle of the parasite in which avian or mammalian hosts are not involved.

There is also the possibility that many of the Yellowstone cutthroat trout stocked in Glacier Park may have escaped downstream via tributaries draining to the Flathead River, possibly en route to Flathead Lake located approximately 100 km southwest of the park. However, the low incidence of hybridization between native trout and introduced Yellowstone cutthroat trout in Glacier Park is attributed primarily to the inability of *S. c. bouvieri* to survive in competition with native trout.

Agreement of Meristic and Electrophoretic Results

Twenty-nine population samples were subjected to both meristic and electrophoretic analyses. Since specimens were individually numbered, direct comparison between the two diagnostic procedures was possible. Very close agreement was observed.

The high degree of concordance between the two methodologies is best illustrated by the Arrow Lake sample. Of 30 specimens collected, two from the lake's outlet stream were identified as *S. c. bouvieri* by both procedures. Of the remaining 28 trout, electrophoretic and meristic character analyses identified two and five specimens, respectively, as *S. c. lewisi* possessing varying degrees of hybrid influence from *S. bouvieri*. Remaining specimens were determined to be *S. c. lewisi* by both methodologies.

Close agreement between meristic and electrophoretic results was observed in this work because the evolutionary separation between *S. c. bouvieri* and *S. c. lewisi* is relatively great (Loudenslager and Gall 1980; Gyllensten et al. 1985). That is, evolutionary distance between the lineages is sufficient for divergence to have occurred at several loci and for measurable physical differences to have occurred. Taxonomic studies comparing other subspecies of interior cutthroat trouts (i.e. *S. c. utah* and *S. c. bouvieri* (Loudenslager and Gall 1980); *S. c. bouvieri* and the undescribed fine-spotted Snake River cutthroat trout (Loudenslager and Kitchin 1979)) have been unable to demonstrate any consistent divergence in gene loci between the subspecies being compared, probably because the period of evolutionary separation between the taxa has not been as great as has been the case for *S. c. lewisi and S. c. bouvieri*. Thus, the two diagnostic procedures may or may not yield similar conclusions, depending upon the degree of evolutionary divergence between the groups being examined. We believe that application of both electrophoretic and meristic character analyses will often yield more useful information than either method used alone. A similar conclusion was reached by Reinitz (1977) and Busack and Gall (1981).

Conclusion

A noteworthy feature of the native trout of Glacier Park is the lacustrine adapted life history of populations inhabiting the park's interior lakes. Relatively small numbers of trout captured in upstream–downstream traps between 1977 and 1980 near the confluence of several park streams with the North and Middle forks, Flathead River (Graham et al. 1980: Fraley et al. 1981), suggest that most trout inhabiting these waters complete their life cycle within the confines of the natal drainage. Elsewhere throughout their remaining range, lacustrine populations of *S. c. lewisi* have been greatly reduced due largely to habitat loss and competition with introduced salmonids. Remnant populations exist mainly in small streams, often only in restricted headwater reaches (Hanzel 1959; Roscoe 1974; Reinitz 1977). Isolated populations of *S. c. lewisi* survive today in some lakes within Banff, Kootenay, and Waterton Lakes national parks, Canada (McAllister et al. 1981), and in several small lakes scattered throughout western Montana (J. Huston, pers. comm.). Thus, Glacier Park appears to represent the largest geographic province containing indigenous populations of lacustrine-adapted *S. c. lewisi* surviving today. As such, they represent potentially important gene pool reserves which could be useful for reestablishment of this unique cutthroat subspecies in portions of its former range.

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Size-Selective Mortality in Young-of-the-Year Yellow Perch (*Perca flavescens*): Evidence from Otolith Microstructure

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Post, J. R., and A. B. Prankevicius. 1987. Size-selective mortality in young-of-the-year yellow perch (*Perca flavescens*): evidence from otolith microstructure. Can. J. Fish. Aquat. Sci. 44: 1840–1847.

Large body size and high growth rates can strongly influence the survival of larval and juvenile fish during periods of intense predation pressure. Experimental evidence suggests that piscivorous fish often feed size selectively, but direct field evidence of size-selective mortality and of the effect of rapid growth on the intensity of size-selective mortality is limited. We used the growth record from otolith microstructure to test the hypothesis that young-of-the-year yellow perch (*Perca flavescens*) that survived their first growing season were the larger and faster growing members of their cohort. Strong size-selective mortality was identified within the first growing season for a slowly growing population. During the same year, a faster growing population showed minimal size-selective mortality. In contrasting the two populations, differences in predator species composition confounded the growth rate size-selectivity relationship, but it was clear that size-selective predation could be intense, favouring the survival of the faster growing individuals within a population.

Une taille importante et un taux de croissance élevé peuvent influer fortement sur la survie des poissons de stades larvaire et juvénile au cours des périodes d'intense prédation. Les données expérimentales portent à croire que les poissons piscivores ont souvent tendance à choisir des proies d'une certaine taille, mais on dispose de très peu d'observations directes sur la mortalité sélective quant à la taille et sur les effets d'une croissance rapide sur l'intensité de cette mortalité. Les auteurs se sont servis des données sur la croissance obtenues de l'examen de la microstructure des otolithes pour tester l'hypothèse voulant que les perchaudes (*Perca flavescens*) nées au cours de l'année qui survivaient à leur première saison de croissance étaient les individus de la cohorte qui étaient les plus gros et dont la croissance était la plus rapide. Une importante mortalité sélective quant à la taille a pu être déterminée au cours de la première saison de croissance des individus d'une population à croissance lente. Au cours de la même année, ce taux de mortalité s'est avéré très faible chez une population à croissance plus rapide. La comparaison de ces deux populations a permis de noter, même si les écarts entre la composition des espèces prédatrices rendaient indiscernable la relation entre le taux de croissance et la sélectivité quant à la taille, que la prédation selon certaines tailles pouvait être intense au sein de la population, ce qui favorisait la survie des individus à croissance plus rapide.

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Body size is an important variable in both competitive (Werner et al. 1983; Persson 1983; Sandheinrich and Hubert 1984) and predator–prey (Nielsen 1980; Werner and Gilliam 1984; Tonn and Paszkowski 1986) interactions within fish communities. Since these are the mechanisms of interaction within and between populations, an understanding of the size-specific intensity of these interactions is necessary to gain insight into the patterns of population and community structure.

Size-selective predation has been demonstrated for coho salmon (*Oncorhynchus kisutch*) feeding on juvenile chum salmon (*O. keta*) (Parker 1971; Healey 1982; Hargreaves and LeBrasseur 1986), walleye (*Stizostedion vitreum vitreum*) feeding on juvenile yellow perch (*Perca flavescens*) (Nielsen 1980), and yellow perch feeding on central mudminnows (*Umbra limi*) (Tonn and Paszkowski 1986). In each of these cases, smaller members of the prey cohort suffered a higher risk of mortality than did the larger members. This differential mortality rate can significantly control prey population structure and abundance (Tonn and Paszkowski 1986).

Large body size can provide a refuge from predation (Werner and Gilliam 1984). Fast growth rates, in reducing the time that the prey are in the more vulnerable size classes, will reduce the impact of predation on the abundance of a cohort. Therefore, body size and growth during early life may have a significant effect on year class strength and subsequent recruitment.

As part of an ongoing study aimed at determining the factors that control recruitment in yellow perch, we tested the general hypothesis that size-selective mortality was important during the first growing season. Since saggital otoliths of juveniles of many fish species contain daily growth records (Campana and

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TABLE 1. Physical and chemical characteristics of Dickie and St. George lakes.

	Dickie[a]	St. George
Area (ha)	93.2	10.3
Mean depth (m)	5.0	5.9
Maximum depth (m)	12.0	14.3
pH	6.0	7.4
Calcium ($mg \cdot L^{-1}$)	2.2	75.2
Chl *a* ($\mu g \cdot L^{-1}$)	5.5	7.9

[a] Data from Ontario Ministry of the Environment, Dorset Research Centre, Dorset, Ont.

Nielson 1985), we tested the validity of this technique and applied it to young-of-the-year yellow perch. Validation involved testing two hypotheses: (1) that otolith microzonation had a daily periodicity and (2) that there was a predictable proportionality between otolith and fish growth. Using this technique, the size of young-of-the-year perch that survived their first growing season was backcalculated and compared with the size distribution observed earlier in their development. This allowed the test of the null hypothesis that the surviving young-of-the-year perch were a random subsample of the population at an earlier time. The analysis was conducted for both a slow- and a fast-growing population, testing the hypothesis that body size and growth rate can affect the intensity of size-selective mortality.

Materials and Methods

Young-of-the-year yellow perch were captured both nearshore and offshore in Dickie (45°09′N, 79°05′W) and St. George (43°57′N, 79°25′W) lakes, Ontario, between June and September 1985. The lakes are morphometrically and chemically dissimilar, Dickie being a dilute Precambrian Shield lake and St. George being an alkaline kettle lake (Table 1). Productivity, as indicated by chlorophyll *a*, is approximately 50% higher in St. George Lake. Both nearshore and offshore habitats were sampled because young-of-the-year yellow perch are known to inhabit both littoral and pelagic zones at different developmental stages during their first growing season (Whiteside et al. 1985). The nearshore zone was sampled with a 30 × 1.6 m beach seine. Offshore fish were captured with a 10-m-diameter, 6.m-deep purse seine. Both nets were constructed of 0.8 × 1.0 mm polyester mesh. Catches were put on ice, counted and subsampled for total length, and then frozen within 10 h for subsequent analysis. Sampling periods were approximately monthly, from June to October 1985.

Saggital otoliths were dissected out under a 4 × dissecting lense, rinsed, and stored in 95% ethanol. Prior to mounting, otoliths were air dried. One otolith from each pair was embedded in a thermoplastic polymer (Crystal Bond 509, Aremco Products Inc.) on a glass microscope slide. Aluminum oxide lapping film (9 to 0.3 μm) was used to grind and polish the mounted otolith. Smaller otoliths were ground to the midplane, proximal surface down on the slide. Larger otoliths were first embedded distal surface down, ground towards the midplane, reheated, turned over, proximal surface down, and ground to the midplane. During the grinding process, regular observations were made with a light microscope at 100–400 × to determine the appropriate extent of grinding. Prior to enumeration of the zonation, completed preparations were soaked in 95% ethanol for 30–45 s and then a drop of 70% glycerol was applied to improve contrast. All microzone counts and radius measurements were made along the posterior axis of the otolith. It was usually necessary to adjust the focal depth to observe all microzones on an otolith. An occular micrometer was used to make the radius measurements at 400–1000 × magnification.

To validate their relative age, otoliths were prepared from 10–11 young-of-the-year perch captured in each habitat in each lake per month. Total microzone counts and radius measurements were made for each preparation. Two steps were involved in testing the size-selection hypothesis. First, intermediate radii from the September preparations from the validation study were measured at 30 microzone increments from the posterior edge. Since only 10 otoliths per habitat per month were prepared for the validation study, the second step involved a larger sample of 30 otoliths, prepared from yellow perch caught in September. Intermediate radii were measured at microzone increments to coincide with the field sampling in Dickie Lake on July 8, 1985, and in St. George Lake on June 24, 1985. On these dates the lengths of the fish in the two lakes were similar (Dickie Lake, 25–45 mm; St. George Lake 27–42 mm). This second larger sample of fish allows for a more robust test of the size-selective mortality hypothesis.

A Kolmogorov–Smirnov two-sample test with a chi-squared approximation was used to compare the backcalculated and field frequency distributions. In cases where sample size was less than 40, this gives a conservative test of the null hypothesis (Siegel 1956).

Results

Validation of the Otolith Method

Microzones were discernible in ground otoliths from young-of-the-year yellow perch collected between June and October in Dickie and St. George lakes. Total microzone counts could be made from the posterior edge to the nucleus in 95% (38/40) and 100% (32/32) of the otoliths prepared from young-of-the-year perch collected during June, July, and August from Dickie and St. George lakes. Darkening of the central portion of otoliths did not permit differentiation of the translucent and opaque zones; therefore, microzone counts could not be made from the edge to nucleus in many of the September and October preparations. Complete counts could be made in 20% (2/10) and 60% (6/10) of September and 40% (4/10) and 0% (0/10) of October specimens.

Three independent microzone counts were done on each otolith prepared. Standard errors of the means of repeated counts were calculated and a mean standard error calculated for the otoliths prepared from each habitat and date for each lake. The mean standard errors on the repeated ring counts were higher for the Dickie than the St. George collections (Table 2). This lower repeatability of microzone counts for the Dickie perch is probably due to their slower growth (Fig. 1) and therefore smaller microzone increments, which increases the probability of reader error. There is no trend of increasing mean standard error with age (Table 2), suggesting that the reader error is not related to the reduced ability to count the microzones near the edge, as growth slows in the fall.

To test the hypothesis that the microzones were produced daily, Julian date was plotted against microzone count. All counts were very close to the 1:1 line which suggests that on

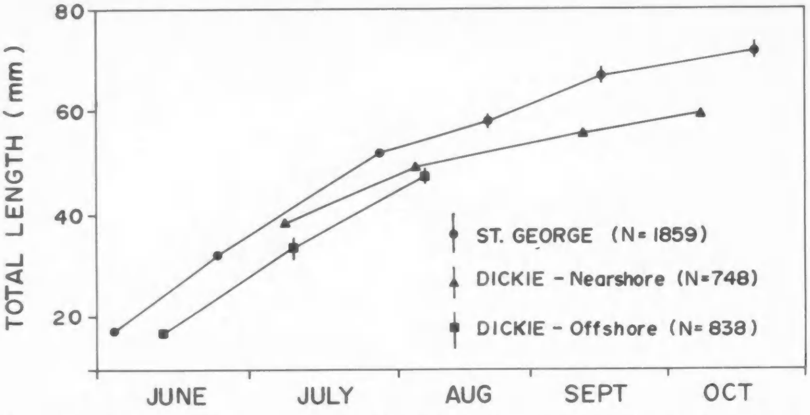

FIG. 1. Growth of young-of-the-year yellow perch in Dickie and St. George lakes. Sample sizes and 95% confidence intervals are indicated.

TABLE 2. Mean microzone count, mean standard error (MSE), and 95% confidence intervals (CI) on repeated counts for otoliths collected nearshore (NS) and offshore (OS) in Dickie and St. George lakes.

Date	Habitat	*N*	Mean	MSE	95% CI
		Dickie			
July 8	OS	10	68.2	2.06	63.5–72.9
July 8	NS	8	69.5	2.71	63.4–75.6
August 6	OS	10	94.5	1.94	90.1–98.9
August 6	NS	10	103.6	2.73	97.4–109.7
September 12	NS	2	133.7	1.91	127.6–139.7
October 8	NS	4	164.3	2.82	156.5–172.2
		St. George			
June 24	NS	10	54.0	0.33	53.2–54.7
July 29	NS	11	83.7	0.47	82.7–84.8
August 23	NS	11	110.1	0.76	108.4–111.8
September 11	NS	6	137.3	0.47	136.1–138.5
October 19	NS	0			

average, the microzones were produced with a daily periodicity in both lakes (Fig. 2). Linear regressions between Julian date and microzone count for Dickie Lake perch caught nearshore and offshore and St. George Lake perch all had slopes not significantly different from 1.00 (*t*-test, $p < 0.05$), also suggesting a 1:1 correspondence of microzones and days.

Regressions of otolith radius to total fish length for Dickie Lake perch caught nearshore and offshore and for St. George Lake (Fig. 3) perch were significant (r^2, $p < 0.05$). Regressions from nearshore and offshore caught perch from Dickie Lake had intercepts that were the same but had different slopes (*t*-test, $p < 0.05$). The range of otolith radii for the offshore data was 436–744 μm, whereas the range for the nearshore data was 458–1070 μm. Since curvilinearity in the data could cause a difference in slopes when the range of variables is different, slopes and intercepts were compared from the August collection, when ranges in otolith radii and fish total length were similar. This comparison showed that there was no difference in the otolith radius versus total fish length relationship for Dickie Lake fish caught in the two habitats (Table 3). This also suggests that for the smaller fish, the otolith radius versus total length relationship may be allometric, as opposed to the iso-

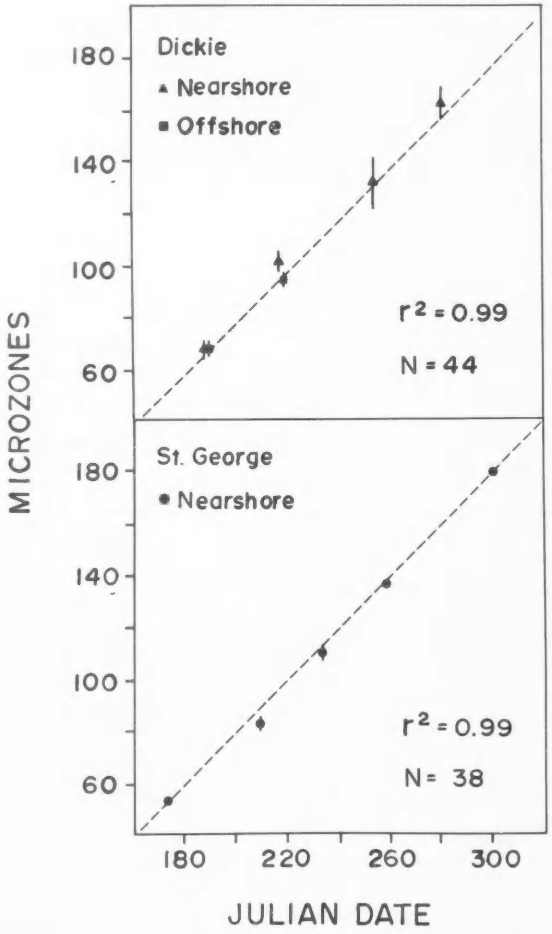

FIG. 2. Mean number of otolith microzones from young-of-the-year yellow perch from Dickie and St. George lakes. Samples sizes, 95% confidence intervals, and correlation coefficients are indicated.

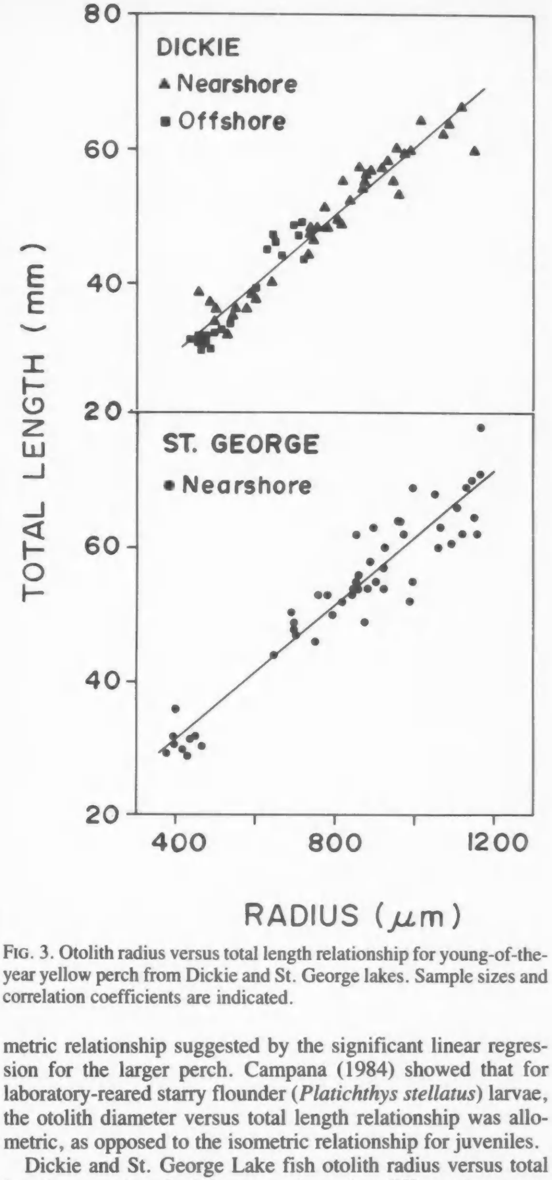

FIG. 3. Otolith radius versus total length relationship for young-of-the-year yellow perch from Dickie and St. George lakes. Sample sizes and correlation coefficients are indicated.

metric relationship suggested by the significant linear regression for the larger perch. Campana (1984) showed that for laboratory-reared starry flounder (*Platichthys stellatus*) larvae, the otolith diameter versus total length relationship was allometric, as opposed to the isometric relationship for juveniles.

Dickie and St. George Lake fish otolith radius versus total length regressions had the same slope, but different intercepts (Table 3). This suggests that for an incremental change in total fish length, the incremental change in otolith radius is the same for both lakes. It also suggests that at a given total length, the otoliths of Dickie Lake perch were larger. Marshal and Parker (1982) showed that fed sockeye (*O. nerka*) salmon fry had a smaller otolith diameter at a given total length than did starved fry. This is analogous to the observed relationship between the fast-growing, presumably higher ration St. George Lake young-of-the-year perch and the slower-growing, lower ration Dickie Lake young-of-the-year yellow perch. In this study, lake pH and calcium concentration had no obvious effect on otolith size, the larger otoliths being from the lake with lower pH and calcium (Table 1). Geen et al. (1985) showed that pH in the range of 5–7 had no effect on otolith size in laboratory-reared chinook salmon (*O. tshawytscha*). A similar observation was recorded by Beamish et al. (1975) who suggested that even though plasma calcium concentrations are maintained by bone deminerilization in fish exposed to low pH, otoliths may not be affected because size is probably controlled by the deposition of the organic matrix rather than calcium carbonate. The observed differences in otolith size – fish size relationships could have a genetic basis, since otolith shape has been used to identify discrete stocks (Casselman et al. 1981; Ihssen et al. 1981).

TABLE 3. Comparisons between regression coefficients using *t*-tests. NS = nearshore, OS = offshore, ns = not significant ($p > 0.05$), *significant ($p < 0.05$).

Comparison	*N*	*a*	*b*
Dickie: NS vs. OS (all data)	60	0.43 ns	2.25*
Dickie: NS vs. OS (August data)	20	1.43 ns	0.27 ns
Dickie (all data) vs. St. George	113	2.57*	0.41 ns

Size Selection

Graphical comparison of total lengths derived from otolith radii from fish caught in Dickie Lake on three sample dates suggests the possibility of size-selective mortality (Fig. 4). The mean backcalculated length in July, from September-caught fish, is larger than is the mean length derived from otoliths collected in Dickie Lake in July. This difference is less distinct in a comparison between the mean backcalculated August length, from September-caught fish, and mean length derived from otoliths collected in August. This suggests that the size-selective mortality was more intense early in the summer. Similar comparisons for St. George Lake caught fish show no such pattern (Fig. 4). This suggests that size-selective mortality may not be important in St. George Lake, at least during the period of this analysis.

A more robust test of the size-selective mortality hypothesis was designed which allows a direct comparison of backcalculated length distributions with length distributions determined from field sampling. Both populations were sampled for length distributions in September 1985. Sampling was conducted both nearshore and offshore but only nearshore samples contained young-of-the-year yellow perch. This is consistent with reported distribution patterns for young-of-the-year of this species in early fall (Noble 1975; Kelso and Ward 1977; Whiteside et al. 1985). A random subsample of 30 fish was selected from the total September catches from Dickie Lake and St. George Lake (Fig. 5) and was used for the otolith analysis. The 1985 St. George Lake cohort of yellow perch was faster growing than was the 1985 Dickie Lake cohort and attained a larger size in September (Table 4; Fig. 5). The otoliths from the subsample of fish were prepared and backcalculations done to coincide with the dates of the early summer field collections which was the period identified by the previous analysis as a time during which size-selective mortality was suggested. In early summer, young-of-the-year yellow perch occupied both the nearshore and offshore habitats in both lakes. This is consistent with the reported distributions for that stage of their early life history (Kelso and Ward 1977; Whiteside et al. 1985).

On July 8, 1985, the Dickie Lake nearshore caught fish had a larger mean length ($t = 12.44$, $p < 0.05$) and a significantly different length distribution ($\chi^2 = 67.46$, $p < 0.05$) than did

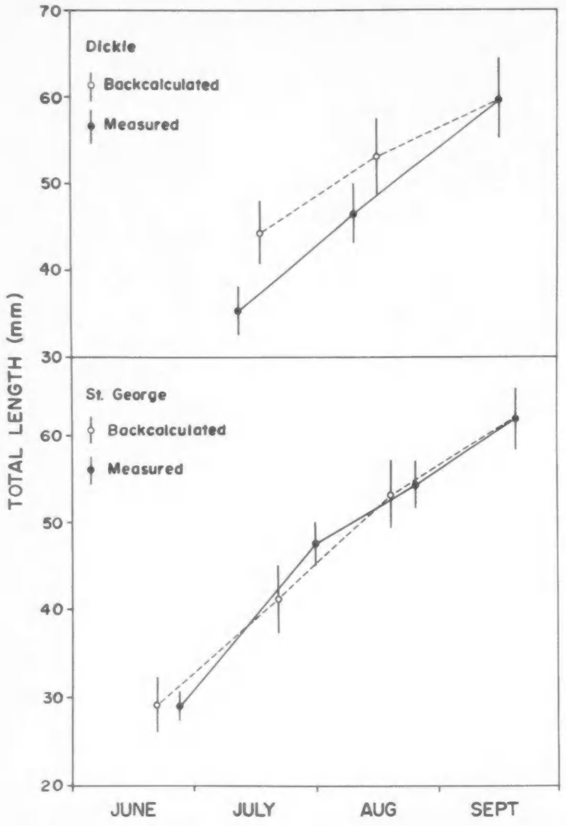

FIG. 4. Backcalculated total lengths from September-caught young-of-the-year yellow perch and total lengths calculated from measured otolith radii from yellow perch caught in Dickie and St. George lakes. 95% confidence intervals are indicated.

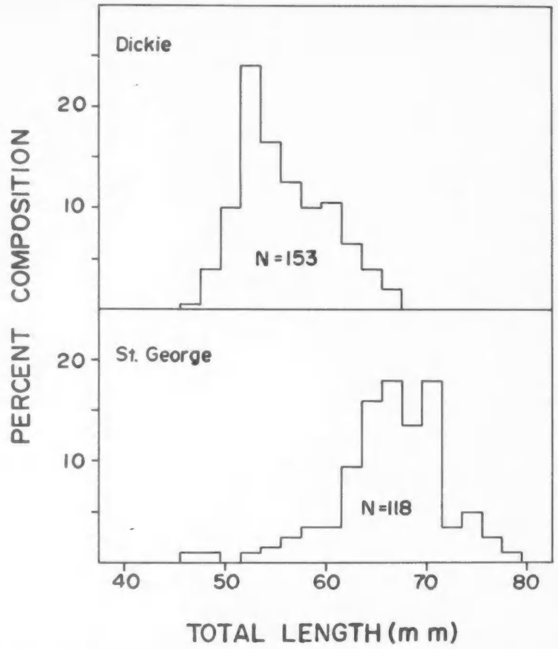

FIG. 5. Length frequency distributions of young-of-the-year yellow perch caught in Dickie Lake on September 12, 1985, and in St. George Lake on September 16, 1985. Sample sizes are indicated.

TABLE 4. Mean total length and growth rate of nearshore-caught young-of-the-year yellow perch from Dickie and St. George lakes.

Lake	Date	Mean total length (mm)	N	Growth rate ($\mathrm{mm \cdot d^{-1}}$)
Dickie	July 8	38.4	74	
	September 12	55.7	153	0.26
St. George	June 24	32.4	66	
	September 16	66.8	118	0.41

the offshore caught fish, although they were not significantly older (Table 2). This is consistent with the hypothesis that the timing of the nearshore migration of young-of-the-year yellow perch is size related. Comparison of the backcalculated length distribution of those fish that survived to September with length distributions of those fish caught nearshore and offshore in July suggests that the smaller 56% of the offshore fish did not survive to September (Fig. 6). The backcalculated length distribution of fish caught in September is significantly different from both the July-caught nearshore ($\chi = 19.67$, $p < 0.05$) and offshore ($\chi^2 = 21.65$, $p < 0.05$) length distributions. The backcalculated length distribution probably represents the amalgamation of the surviving nearshore fish, which suffered no size-selective mortality, and the survivors of the offshore fish, which suffered severe size-selective mortality between July and September. An alternative hypothesis is that the group of fish that was distributed offshore in July was much less abundant than was the nearshore group. If this were the case, it would not be surprising if they were not represented in a relatively small sample taken in September. Two lines of evidence suggest that this was not the case. First, the density of offshore fish was approximately four times that of the nearshore fish in July (Table 5). Since 61% of the surface area of Dickie Lake is considered offshore (>4.0 m depth), the absolute abundance was certainly greater offshore than nearshore in July. Second, the shape of the backcalculated length distribution suggests that the apparent loss of offshore fish is not a sample size artifact (Fig. 6). To account for the shape of the distribution for the smaller sizes, a portion of larger sized offshore fish must have been added to the portion of the cohort already nearshore in July. This is consistent with the hypothesis that the smaller half of the offshore fish did not survive and that the larger remaining offshore fish in July did survive and were distributed in the nearshore area by September. It cannot be determined from the data available whether the size-selective mortality took place before or after the nearshore migration of those fish that were offshore in July.

A graphical comparison of the St. George Lake backcalculated mean lengths with the observed mean lengths suggests no size-selective losses (Fig. 4). Size frequency data suggest that 5% of those fish caught nearshore in June were not represented in the catch of September survivors (Fig. 7). Statistical analysis suggests that there was a significant difference between backcalculated length frequency of survivors and the July nearshore

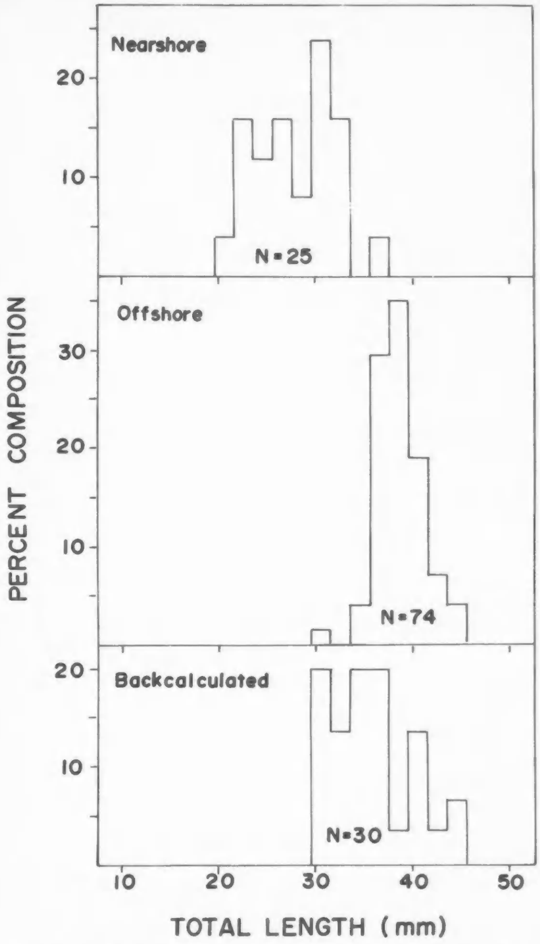

FIG. 6. Length frequency distributions of young-of-the-year yellow perch caught nearshore and offshore in Dickie Lake on July 8, 1985, and backcalculated to that date from yellow perch caught on September 12, 1985. Sample sizes are indicated.

fish ($\chi^2 = 9.54$, $p < 0.05$) but not a significant difference with the July offshore-caught fish ($\chi^2 = 0.35$, $p > 0.05$). This small, though statistically significant difference probably has little biological significance.

Discussion

In this study, strong size-selective mortality was identified in one of the two populations examined. Growth history data recorded in the otoliths of individuals surviving their first 4–5 mo of life in Dickie Lake suggested that those that survived had attained a larger body size by early summer at approximately 2 mo of age. Growth history data for young-of-the-year yellow perch from St. George Lake did not show strong size-selective losses of the smaller members of the cohort. Two major differences between the fish communities of the two lakes may explain the different results of the growth history analysis. First, the size and growth rates of the Dickie Lake cohort were significantly lower than were those of the St. George Lake

TABLE 5. Mean catches (standard deviations) of young-of-the-year yellow perch in Dickie Lake in nearshore (NS) and offshore (OS) habitats.

Date	Habitat	N	Catch·m^{-2}	(SD)
July 8	NS	4	0.25	(0.16)
	OS	6	1.02	(2.51)
September 12	NS	5	0.45	(0.85)
	OS	6	0	

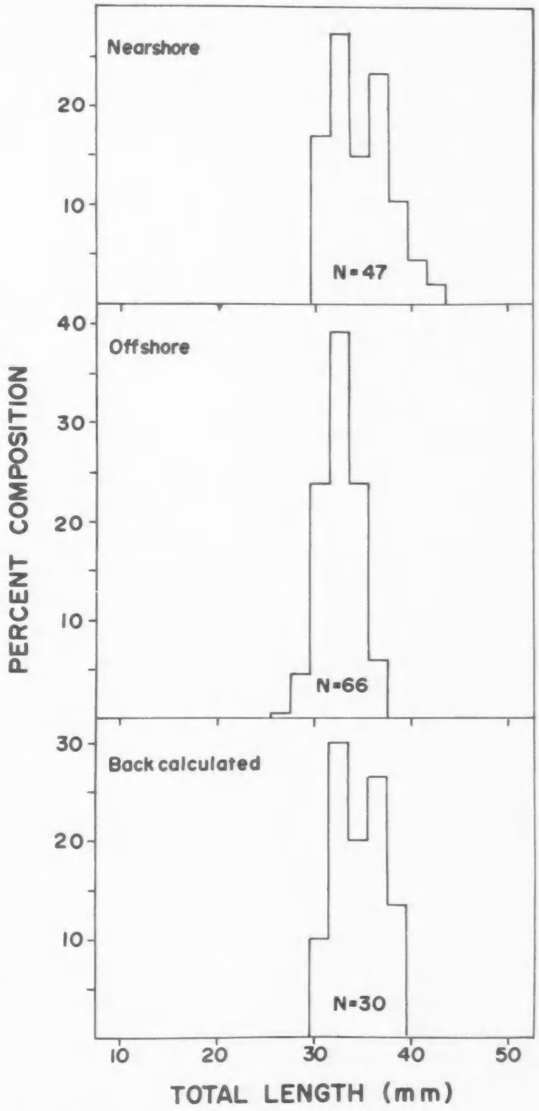

FIG. 7. Length frequency distributions of young-of-the-year yellow perch caught nearshore and offshore in St. George Lake on June 24, 1985, and backcalculated to that date from yellow perch caught on September 16, 1985. Sample sizes are indicated.

cohort. Thus, Dickie Lake fish remained in size classes that were susceptible to predation for longer periods. Second, the predator guild found in the two lakes was also different. Dickie Lake has populations of small-bodied largemouth bass (*Micropterus salmoides*), smallmouth bass (*Micropterus dolomieui*), and adult yellow perch (Harvey and Lee 1981). The size-limited gape and pursuit efficiency of these small predators might be expected to restrict predation impacts to small-bodied prey (Werner and Gilliam 1984). The predators of St. George Lake are dominated by a population of large-bodied northern pike (*Esox lucius*) and also have small populations of small-bodied largemouth bass and adult perch. The primary prey item of the northern pike in the summer of 1985 was young-of-the-year yellow perch. A population of large-bodied northern pike would not be expected to be gape limited, and therefore, size selection should not be expected. With the data available, it is not possible to separate the impacts of these two between-lake differences, but both must have contributed to the observation that size selection took place in Dickie Lake, but was not observed in St. George Lake.

Size-selective mortality has also been demonstrated for juvenile chum salmon in laboratory experiments (Parker 1971) and in large enclosures in the field (Hargreaves and LeBrasseur 1986) and has been suggested from scale measurements of wild-captured chum salmon (Healey 1982). These authors suggested that the probable cause of size-selective mortality in chum salmon in the sea was predation by coho salmon and that its intensity is probably related to chum size and growth rate and coho size.

When evidence of size-selective mortality is presented, size and growth rate are implicated as variables that can mediate the impact of losses to predators and therefore year class strength. Optimal foraging theory suggests that for a given size of predator, there is an optimum prey size that maximizes net energetic gain (Werner and Gilliam 1984). Therefore, from the standpoint of the survival of the prey, a larger body size might be expected to reduce the risk of capture by predators. However, in size-structured populations, all species develop ontogenetically through a series of stages which are size related (Werner and Gilliam 1984). This suggests that during early life history, individuals grow through size classes that are vulnerable to a guild of predators, and the rate of growth through these vulnerable size classes determines the total predation risk. The data presented here support this prediction that the smaller bodied, slower growing young-of-the-year perch had a relatively higher mortality rate.

Since growth rate is a function of prey size and abundance, the intensity of size-selective mortality may be controlled by the next lower trophic level. There are some data in the literature to illustrate this point for young-of-the-year yellow perch. The combined results of several studies done at Oneida Lake, New York, suggest that the quantity and quality of prey has an indirect but significant effect on the year class strength of young-of-the-year perch. The abundance of *Daphnia pulex* during the spring and summer controls the growth rate of young-of-the-year yellow perch and size attained by the fall (Noble 1975; Lin 1975). Neilsen (1980) showed that cohorts that had a greater mean length survived walleye predation better through their first summer and fall. Also, Tarby (1974) showed that the intensity of cannibalism on young yellow perch by adults in Oneida Lake was negatively correlated with length. Therefore, the abundance of *D. pulex*, by controlling young-of-the-year yellow perch growth rates, was a significant factor in the development of cohort strength and in reduction of mortality due to predation. Since abundant cohorts of young-of-the-year perch can reduce or eliminate *D. pulex* populations (Noble 1975; Mills et al. 1985), density-dependent factors may be more important in determining year class strength in yellow perch than is predation.

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Food-Induced Granulomatosis in European Smelt, *Osmerus eperlanus*

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Anders, K., and H. Möller. 1987. Food-induced granulomatosis in European smelt, *Osmerus eperlanus*. Can. J. Fish. Aquat. Sci. 44: 1848–1854.

Many European smelt, *Osmerus eperlanus*, in tributary rivers to the North Sea are affected by granulomatosis in the buccal cavity. The granulomas are caused by spines and leg fragments of amphipods, an important food item of smelt. Granuloma prevalence increases with increasing fish size. The rate of increase slows down in very large fish, probably as an effect of changed feeding habits. Fungi and virus-like particles in the granulomas are assumed to be secondary invaders. Granulomas are occasionally found in Atlantic cod, *Gadus morhua*, and flounder, *Platichthys flesus*, also.

Beaucoup d'éperlans d'Europe (*Osmerus eperlanus*) des tributaires de la mer du Nord présentent une granulomatose de la cavité buccale. Les granulomes sont causés par des fragments d'épines et de pattes d'amphipodes, lesquels constituent une fraction importante du régime alimentaire des éperlans. La prévalence des granulomes augmente avec la taille des poissons. Le taux d'augmentation diminue chez les très gros éperlans, probablement en raison du changement de régime alimentaire. Les champignons et particules d'aspect viral présents dans les granulomes seraient des germes secondaires. Des granulomes sont aussi parfois observés chez la morue de l'Atlantique (*Gadus morhua*) et le flet (*Platichthys flesus*).

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In 1981–82 a series of surveys on fish stocks and fish diseases was carried out on the lower Elbe River (Möller 1984a). A second survey series in 1984–86 gave us the opportunity to study selected items in greater detail. The project was aimed at evaluating the effects of environmental parameters, natural and man-made, on fish health.

Despite pollution problems, heavy shipping, and shore regulation (Riedl-Lorjé and Gaumert 1982), the lower Elbe is still rich in fish life. In stow net catches made in the freshwater region between Brunsbüttel and Hamburg, European smelt, *Osmerus eperlanus*, is the dominant species by number as well as by weight (Möller 1984a). Adult smelt are assumed to inhabit shallow areas of the Waddensea during most of the year. In February the spawners gather in large shoals in the mouth of the Elbe from where they start their migration upstream. The main spawning grounds seem to be located along the southern shores between Hamburg and Stade, probably also upstream from Hamburg (Dieckwisch 1987).

During our surveys, smelt proved to be the local species with the largest variety of disease symptoms and lesions, including skeletal anomalies, ulcer-like and tumor-like lesions, and a number of highly pathogenic endoparasites. One of the "new" diseases encountered was the "pharyngeal granuloma" in the buccal cavity of smelt (Möller 1984b). It had not been described before by Lillelund (1961) who studied the biology of the local population, by Jarling (1982) during his investigation on smelt helminths, or by Köhler and Hölzel (1980) who studied the health condition of smelt in the Elbe estuary. It had also not been reported during parasitological studies of the European smelt from Finnish lakes (Voigt 1975) or Lake Ladoga, USSR (Voronin 1981), or from the American smelt, *O. mordax*, from landlocked populations in Newfoundland (Threlfall 1981), lakes in Quebec, Canada (Delisle 1972; Fréchet and Dodson 1983), Lake Erie and Lake Ontario, Canada (Chen and Power 1972; Nepszy et al. 1978), and estuaries in New Hampshire, USA (Haley 1953).

The purpose of the present paper is to describe the etiology of the granulomatosis and to examine fluctuations in the prevalence of the lesion.

Materials and Methods

Sampling

The present study was carried out in the lower reaches of four German rivers emptying into the North Sea (Fig. 1). One reference sample was taken from the Schlei, a shallow fjord in the western Baltic Sea. The majority of samples are from the lower Elbe between Hamburg and Cuxhaven (Fig. 1). Most fish were taken from commercial gear: from stow nets in the Ems, from dip nets in the Weser, from shrimp trawls and stow nets in the Elbe, from fyke nets in the Eider, and by electrofishing in the Schlei Fjord.

A pilot project was carried out in the lower Elbe River from October 1981 to November 1982. On a monthly basis, 12 stations were surveyed by stow nets, 8 stations by shrimp trawls, and 9 stations by bottom trawl. The presence of granulomas was registered as part of a general survey on fish diseases in the river (Möller 1984a).

Unless otherwise stated, all following data concerning the Elbe are from the main project, carried out from February 1984 to July 1986. All samples were investigated by the same person

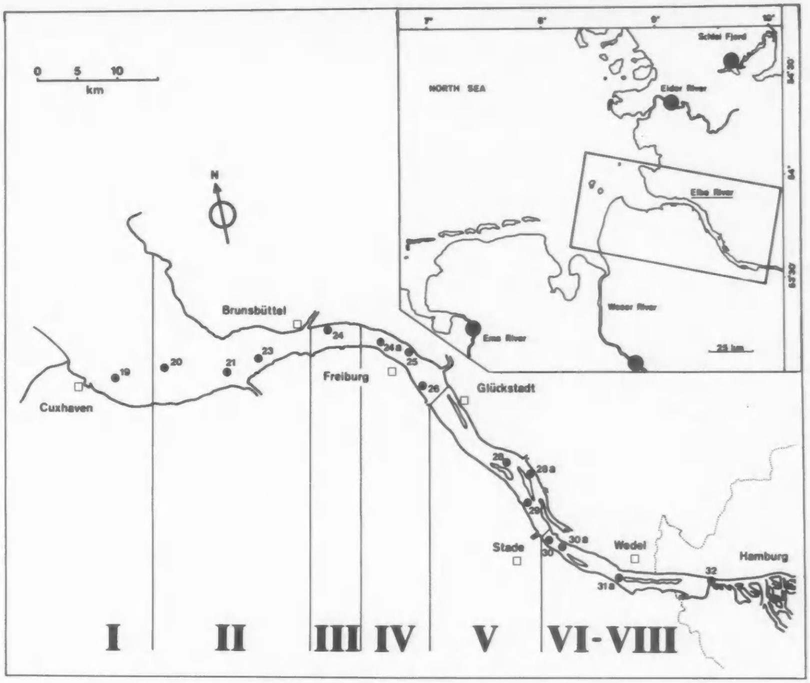

FIG. 1. Study area and sampling stations, February 1984 – July 1986.

to minimize mistakes due to individual habits of the investigator. Altogether, 29 706 (1981–82) and 12 838 (1984–86) smelt from the Elbe, 501 from the Ems, 1873 from the Eider, 38 from the Weser, and 341 from the Schlei were examined.

Examination

Immediately after capture, all fish were examined macroscopically for the presence of granulomas in their buccal cavity, on the gill arches, and on the insides of the opercula. Total length was determined to the nearest centimetre below (i.e. 6.9 cm reported as 6 cm). Condition factor was calculated as $w(100)/l^3$. Subsamples were frozen to −20°C and studied later in the laboratory to determine granuloma size and location within the buccal cavity and to detect granulomas in internal organs. Granulomas in the body muscle were detected by gross examination of the body surface. The relatively transparent skin of smelt permits the discovery of nematodes and granulomas in superficial muscle layers. Granuloma volume v was determined by measuring the diameter d and calculating $v = 0.75\pi \cdot (0.5d)^3$.

Squeeze preparations of buccal granulomas from frozen juvenile smelt were produced for light microscopy by placing the whole granuloma together with a drop of physiological saline between a slide and a coverslip.

For histology, samples of fresh granuloma tissue were fixed immediately after capturing an affected fish in chilled Bouin's solution for about 1 d and then transferred into 70% ethanol. In the laboratory, samples were dehydrated in a graded ethanol series, sectioned at 5 μm, and routinely stained with Mayer's haemalum and erythrosin. The Grocott-Gomori stain (Grocott 1955) was the method of choice for the demonstration of fungal structures.

For transmission electron microscopy, samples were fixed onboard in 2.5% glutaraldehyde in phosphate buffer (0.1M, pH 7.4) for 2 h at approximately 4°C, washed three times in chilled phosphate buffer, and postfixed in phosphate-buffered 1% osmium tetroxide for another 2 h at approximately 4°C. For transport and storage, samples were transferred into 70% ethanol. In the laboratory the tissue samples were dehydrated in a graded pure ethanol series, treated with propylenoxide, and embedded in araldite. Ultrathin sections were cut using a Reichert-Jung OM 3 ultramicrotome, stained with uranyl acetate and lead citrate, and examined in a Philips EM 300 transmission electron microscope.

Thirteen mouth granulomas of 1–5 mm in diameter from different hosts captured during May–September 1984 were taken for mycological and histological examination. Tissue samples were cut into two halves, one of them being processed for histology and the other for mycology. Mycology processing was carried out under sterile conditions. Tissue halves were cut into small distinct pieces of about 1 mm^3 which were plated onto an agar consisting of yeast extract (1 g), D-(+)-glucose (5 g), and agar (12 g) in 1 L of sterile seawater from the North Sea. The seawater was diluted to establish estuarine conditions of about 16‰ salinity. Plates were incubated at room temperature and were checked at irregular intervals for possible growth using a stereomicroscope. Maximum incubation time was 11 mo for one granuloma.

Results

Etiology

Three types of granulomas were identified, one occurring in the buccal cavity, one in the somatic muscle, and a third in stomach wall and liver. This work covers only the first type.

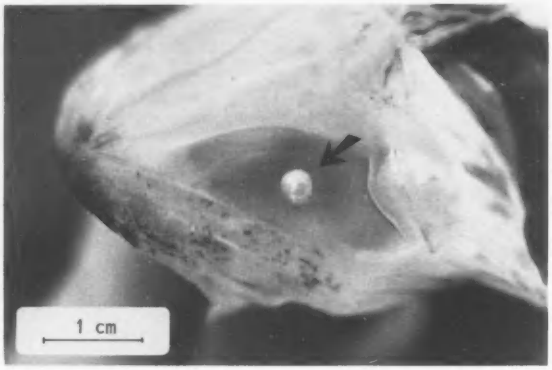

FIG. 2. Typical pearl-like buccal granuloma in the integument of the lower jaw.

The typical buccal granuloma is globular with a pearl-like appearance (Fig.2). The smallest stage that was discovered during the routine survey with the naked eye measured 0.1 mm; the largest one was 8.0 mm in diameter. Granulomas are predominantly located in the thin integument of the lower jaw, the lateral parts of the buccal cavity, and on the gill arches (Fig. 3).

Squeeze preparations of young buccal granulomas (0.1–2.0 mm in diameter) from 7–12 cm Elbe smelt in May 1985 revealed in 124 out of 126 cases the presence of crustacean remnants in the centers of the granulomas (Fig. 4B–4D). The remnants, whenever an identification was possible, were shown to be spines, spine fragments, fine bristles, or even legs of the amphipod *Gammarus zaddachi* (Fig. 4A).

All buccal granulomas showed a similar and characteristic histopathology. They consisted of concentric masses of fibrous encapsulation tissue with central necrotic areas (Fig. 5A). Fungal stages could be demonstrated in most of the 60 mouth granulomas (0.5–6.0 mm in diameter) and 10 of the 11 muscle granulomas examined (Fig. 5B and 5C). They were tentatively classified as belonging to the *Deuteromycotina* (D. Alderman, Fish Diseases Laboratory, Weymouth, England, pers. comm.). Spore-like structures were found in 87% and hyphae in 25% of the buccal granulomas studied. Large granulomas were more frequently interspersed with hyphae than small ones. Several attempts to grow the organism in vitro failed.

Transmission electron microscopy revealed the presence of virus-like particles within the central necrotic areas of one of the 13 buccal granulomas examined (Fig. 5D). The average size of these particles was 20–25 nm, being within the size range of the Picorna- and Parvoviridae.

No significant differences were evident when the condition factors of 17–19 cm Elbe smelt without ($n = 211$, $\bar{x} = 0.437$, $s = 0.056$) and with buccal granulomas ($n = 70$, $\bar{x} = 0.441$, $s = 0.054$) were compared (April–July 1984).

Dynamics of Buccal Granuloma Prevalence

The smallest smelt affected by a buccal granuloma was 7 cm total length. The prevalence (percentage of animals in the sample affected), the mean intensity (average number of granulomas per affected fish), and the mean total volume of all granulomas per infected fish increased with increasing fish length (Table 1). The same observation is made when the granulomas are regarded separately for small, medium, and large types (Fig. 6). The frequency distribution of smelt with different numbers of granulomas is more dispersed in larger fish (Fig. 7).

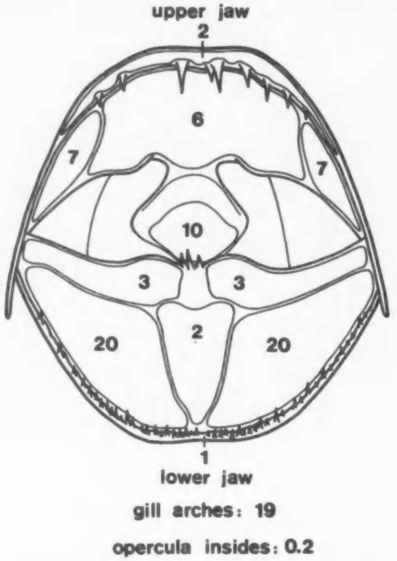

FIG. 3. Distribution (%) of 954 granulomas identified in the buccal cavity of 250 Elbe smelt, studied in March 1985.

The greatest increase in granuloma prevalence takes place from 13- to 15-cm-long smelt, going up from 13.9 to 34.3%. The slope of the curve slows down in larger fish (Fig. 8). In smelt of 25–27 cm the prevalence reaches 51.5%.

No significant differences at the 5% level were observed between granuloma prevalences of 107 female smelt of 17–19 cm (47.7%) from March 1985 and 84 male smelt (42.9%) of the same size range and from the same samples.

In the 13–15 cm group, buccal granulomas in Elbe smelt occurred three times more frequently than in smelt from the Ems and the Eider. In 17–19 cm Elbe smelt the prevalence was still twice as high as in smelt from the other two rivers (Table 2). Two years earlier, in April 1984, only 3.3% of 112 smelt (13–15 cm) from the Schlei and none of 38 smelt (13–19 cm) from the Weser were found with granulomas. During a pilot study on the Weser in summer 1980, 2.9% of smelt larger than 13 cm were affected. Smelt from the Elbe showed a significantly larger mean granuloma volume ($5.7\ \text{mm}^3$, $n = 72$, March 1985) than smelt from the Ems River (3.5mm^3, $n = 62$, April 1986).

In May 1985 the granuloma prevalence of a total of 2111 smelt of 7–10 cm was 5.8% on average in the study area with values above average in the marine and brackish regions I–III (Table 3).

Among 265 flounder, *Platichthys flesus* (15–25 cm), and 145 Atlantic cod, *Gadus morhua* (8–20 cm), from the Elbe estuary, only two flounder (20 and 24 cm) and one cod (12 cm) carried small nodules on the inner side of their opercula. Histology confirmed that they were true granulomas. Fungal growth was not involved.

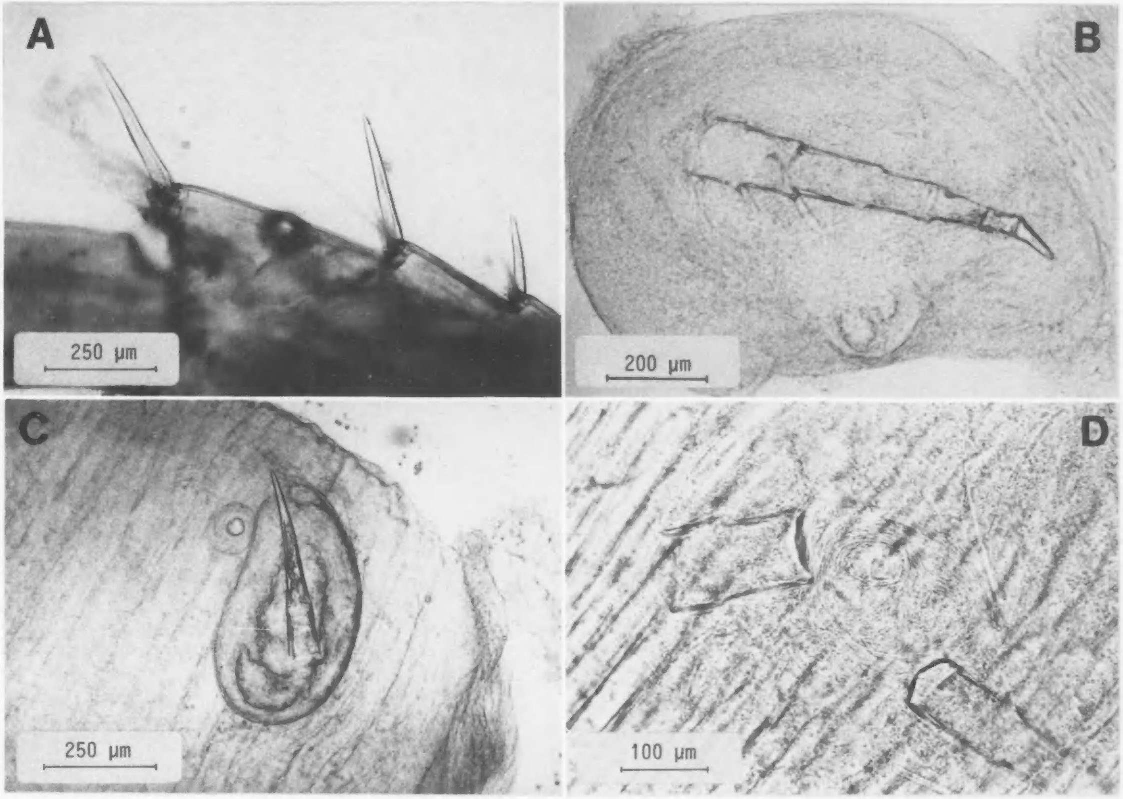

FIG. 4. (A) Part of a *Gammarus zaddachi* leg; (B) leg and (C) spine fragment in the centers of young buccal granulomas from Elbe smelt; (D) beginning granuloma formation at the site of spine penetration.

TABLE 1. Prevalence, mean intensity, and mean volume of buccal granulomas of Elbe smelt in relation to host length (number of fish examined in parentheses).

	Prevalence (%)	Mean intensity	Mean total volume (mm^3)
Date of investigation	Nov. 1984–July 1986	Mar.–May 1985	Mar.–May 1985
Fish length (cm)			
7–10	6.0 (2212)	1.1 (105)	0.4 (105)
11–15	21.3 (1616)	2.5 (94)	1.2 (94)
16–20	39.7 (3337)	3.1 (164)	4.2 (164)
21–25	43.7 (1548)	5.4 (73)	8.1 (73)

Discussion

As far as we are aware, this is the first description of a fish granuloma mechanically induced by food organisms. Further investigations may show that buccal granulomatosis is a common lesion in a number of fish species in various coastal waters, not detected until now due to the lack of specific investigations. Members of the family Osmeridae occur along nearly all coasts north of approximately 40°N (Nelson 1976), and amphipods are common food items of most fish species inhabiting these areas. As documented during this local study, smelt is most susceptible to mouth granulomatosis, but because cod and flounder are occasionally affected, these granulomas may be widespread in other species.

Buccal granulomas of Elbe smelt are tissue reactions to the "injection" of cuticular fragments mainly of the amphipod *Gammarus zaddachi*. This is supported by the finding of such cuticular fragments in nearly all granulomas and by the characteristic development of granuloma prevalence in different

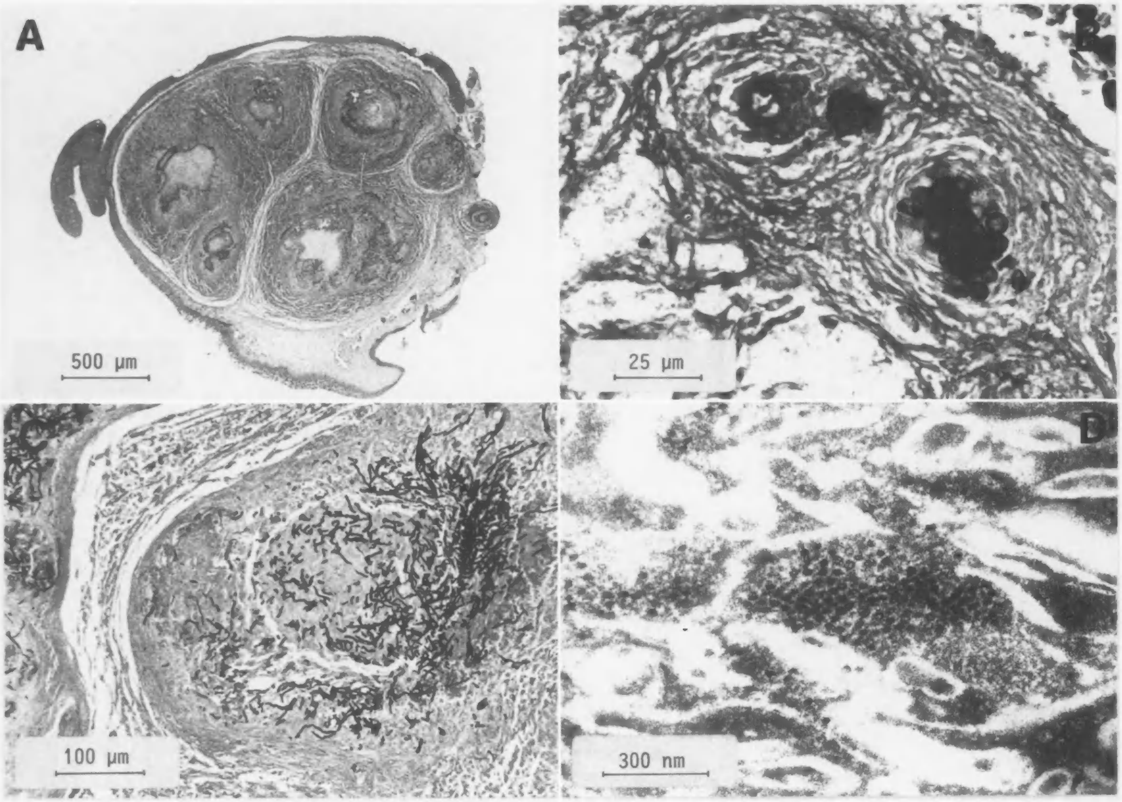

FIG. 5. (A) Typical concentric masses of fibrous encapsulation tissue with central necrotic areas; (B) fungal spores (?) in a buccal granuloma (Grocott–Gomori stain); (C) mass occurrence of fungal hyphae in a granuloma (Grocott–Gomori stain); (D) virus-like particles in a central necrotic area of a granuloma.

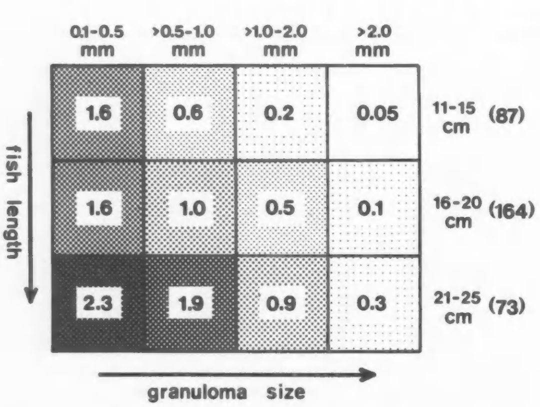

FIG. 6. Increasing intensities in four size groups of altogether 1134 buccal granulomas from 324 Elbe smelt in relation to increasing host size (March 1985, stations 19–30a).

size groups of smelt. Smelt start feeding on amphipods at a total length of 5 cm and the role of amphipods as food items increases up to a length of 13 cm. Larger smelt preferably feed on their own fry and on brown shrimp, *Crangon crangon* (Lüchtenberg 1986). Accordingly, the granuloma prevalence rapidly increases from 7 to 15 cm in length, while the rate of increase slows down in larger fish (Fig. 8). The delay between the first uptake of amphipods at 5 cm and the first occurrence of granulomas at 7 cm might well reflect the time necessary for the development of visible granuloma stages. Figure 6 documents that the formation of new granulomas is possible in all size groups of fish. Other food items with spiny body extrusions similar to that of gammarids might cause similar lesions in smelt.

TABLE 2. Prevalence of buccal granulomas in two size groups of smelt from the lower reaches of three German rivers in April–May 1986.

	Elbe	Ems	Eider
13–15 cm			
No. examined	66	59	117
Prevalence (%)	31.8	6.8	12.0
17–19 cm			
No. examined	156	188	191
Prevalence (%)	52.6	26.1	19.4

The role of fungi and virus-like particles found in the granulomas is largely unknown. We have classified them as secondary invaders. The finding of spore-like structures that can be interpreted as propagative stages of fungi opens the specula-

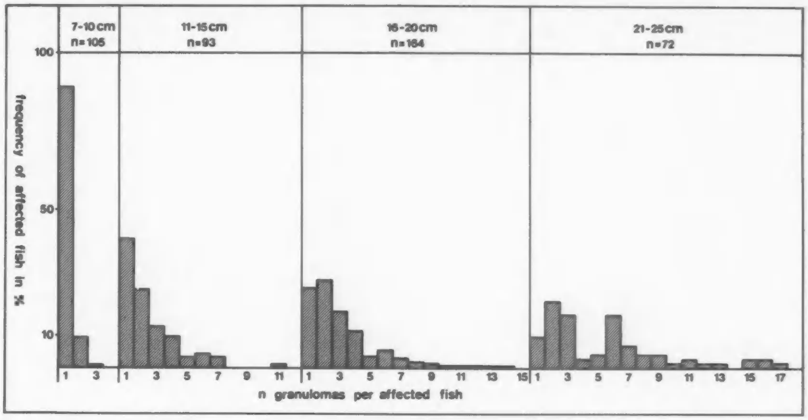

FIG. 7. Frequency distribution of Elbe smelt with different numbers of buccal granulomas in relation to fish length ($n = 434$, March 1985, stations 19–30a).

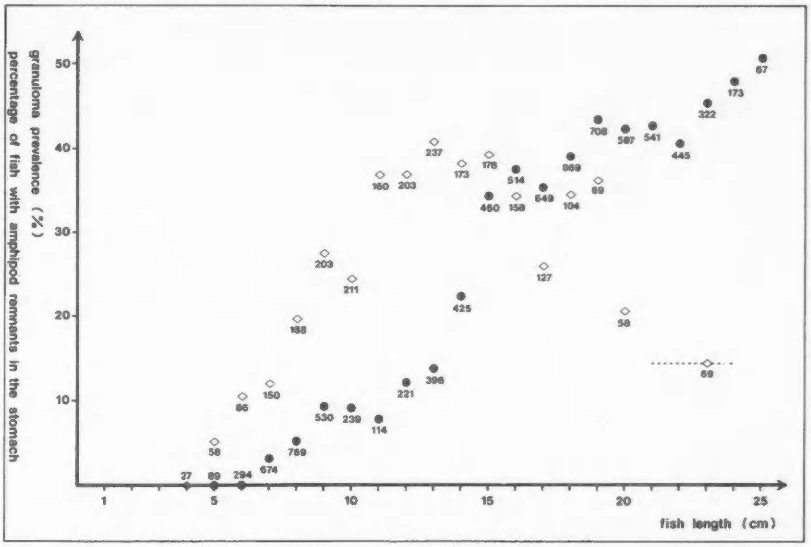

FIG. 8. Relationships between fish length, granuloma prevalence, and importance of amphipods as food organisms with number of fish examined. Circles: prevalence of buccal granulomas in Elbe smelt from all samples ($n = 9128$, November 1984 – July 1986, stations 19–32); diamonds: percentage of Elbe smelt with amphipod remnants in the stomach (calculated from Lüchtenberg 1986) ($n = 2459$, March–August 1985, stations 19–29).

TABLE 3. Prevalence of early granulomas in juvenile smelt (7–10 cm) from different regions (I–VIII) of the lower Elbe in May 1985. Data are length adjusted according to the method of Möller (1981).

	I	II	III	IV	V	VI–VIII
No. examined	136	407	98	574	625	271
Prevalence (%)	6.4	6.3	8.5	5.4	3.5	5.0

tion whether this parasite contributes to the growth of granulomatous tissue and whether it is responsible for the formation of daughter granulomas in the host. One can also speculate that the relatively high granuloma prevalence and the relatively high mean granuloma volume in smelt from the Elbe in comparison with fish from reference areas may be due to the relatively high sewage load of the Elbe River.

The finding of fungi in granulomatous lesions of fish is not uncommon (reviews in Neish and Hughes 1980; Alderman 1982; Lauckner 1984), but only one observation of viruses in granulomas has been reported: Moser et al. (1986) described a granulomatous lesion in sablefish, *Anoplopoma fimbria*, containing acid-fast bacilli and retrovirus-like particles about 55 nm in diameter.

The interpretation of seasonal and regional variations in granuloma prevalence of Elbe smelt is problematic. Present data indicate that such variations are low, if any. From October 1981 to November 1982 the monthly granuloma prevalence in 14-cm-long smelt varied between 3.9 and 9.5% ($\bar{x}$ = 6.0%) with no evident seasonality. A slightly higher prevalence on average of the sampling period was found in the central estuarine regions II and III (6.8%) as compared with the outer estuarine region I (5.7%) and the freshwater regions IV–VIII (6.0%) (Möller 1984b). Similar characteristics were found during the present study where the average granuloma prevalence in 7–10 cm smelt in May 1985 was 7.4% in regions II and III, 6.4% in region I, and 4.6% in regions IV-VIII. No satisfying explanation for these regional differences can be given except that the low prevalence in region I may be due to the relatively low significance of amphipods as food items in that area (Möller 1984a).

We can say nothing of long-term variations in prevalence. An increase in prevalence from 1981 to 1985 as recorded in our data is attributed to increased training of the investigator. We assume that similar problems may also occur during other surveys and recommend that future studies on the epidemiology of fish diseases, including tumors, granulomas, and virus- and protozoa-induced cell hypertrophies, should state clearly the minimum size of lesions recorded.

Acknowledgements

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Ecology and Growth of Postlarval and Juvenile Lobster, *Homarus americanus*, off Îles de la Madeleine (Quebec)

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Hudon, C. 1987. Ecology and growth of postlarval and juvenile lobster, *Homarus americanus*, off Îles de la Madeleine (Quebec). Can. J. Fish. Aquat. Sci. 44: 1855–1869.

Lobsters, *Homarus americanus* were studied in various habitats off Îles de la Madeleine, Quebec, from June to October 1985. Density, biomass, and size structure were estimated quantitatively using underwater corrals (40 m^2) on rocky bottoms or transects (200 m^2) on sandy bottoms and eelgrass beds. On the basis of behavioural observations during capture, lobsters were divided into postlarvae (<25 mm carapace length, CL), juveniles (25–76 mm CL), and adults (>76 mm CL). Density, biomass, and size structure were closely related to the coarseness of the bottom. Density and biomass were greater and mean size was larger on algae-covered boulders than on bare stones. Postlarval and juvenile lobsters were most numerous on shallow rocky bottoms where high densities (0.8–3.8 ind$\cdot$ m^{-2}) of small lobsters (mean size from 23.8 to 35.5 mm CL) were captured. Low density of juveniles and adults occurred on sand covered with eelgrass. No resident lobsters were found on bare sand. Temporal variation of postlarval density was synchronous at the three stations investigated. Postlarval density decreased during the summer until the settlement of planktonic postlarvae in mid-August. Moulting activity occurred throughout the summer, with a peak in early July. Mean size values for stages V–XIV were fitted to the cumulated size distribution and were used to calculate the percent increment for each moult stage (growth factor) and the expected duration of each stage. The 2-mo duration of the stage IV settlement period could result in a size range of 6–21 mm CL (mean size 14.5 mm CL, stage VIII) for a cohort by the end of its first season of benthic growth. The transition from planktonic to benthic life was characterized by a decrease of the growth factor at stage V, which was subsequently compensated at stages VI and VII by high growth factor values. In the first three growth seasons, spring and summer moults exhibit higher growth factor values than fall moults. These seasonal variations result in regular oscillations of the growth factor dampening progressively between stages V and XIV, instead of the smooth decline hypothesized in previous studies. A combination of factors, such as cryptic behaviour, high substrate selectivity, gradual settlement over the late summer months, and variability in growth factor, reflect the very fine tuning of the lobster's early benthic life stages to the constraints of its physical environment.

Les homards, *Homarus americanus*, provenant de différents habitats des Îles de la Madeleine (Québec) ont été étudiés de Juin à Octobre 1985. La densité, la biomasse et la structure de taille des homards ont été évaluées au moyen d'enclos sous-marins de 40 m^2 sur les fonds rocheux, ou de transects de 200 m^2 sur les fonds sablonneux et les prairies de zostère. Des observations comportementales au cours de la capture ont permis de diviser les homards en postlarves (<25 mm de longueur de carapace, LC), juvéniles (25–76 mm LC) et adultes (>76 mm LC). La densité, la biomasse et la structure de taille étaient reliées de près à la taille des matériaux benthiques. Les blocs colonisés par des laminaires supportaient principalement des juvéniles, tandis que les pierres nues abritaient surtout des postlarves. Les densités naturelles maximales (0.8 à 3.8 ind$\cdot$m^{-2}) de postlarves et de juvéniles (taille moyenne de 23.8 à 35.5 mm LC) étaient localisées aux sites rocheux peu profonds. Le sable n'abritait pas de homards résidents quoique de faible densités aient été observées dans les prairies à zostère. Les variations temporelles de densité des postlarves étaient synchrones aux trois stations étudiées, déclinant au cours de l'été jusqu'à la déposition benthique des postlarves planctoniques à la mi-août. La mue est demeurée active tout l'été, débutant par un pic au début de juillet. Les valeurs moyennes de tailles des stades V à XIV ont été calculées d'après la distribution de taille cumulée des captures, ce qui a permis de calculer le pourcentage d'accroissement à la mue (facteur de croissance) ainsi que la durée d'intermue pour chaque stade. Au cours des trois premières saisons de croissance, les mues printanières et estivales engendrent un facteur de croissance supérieur aux mues automnales. Ces variations saisonnières induisent des oscillations régulières du facteur de croissance allant en s'atténuant entre le stade V et XIV, contrairement au déclin graduel attendu. La durée de la période de déposition larvaire pourrait induire un gradient de taille de 6 à 21 mm LC (taille moyenne 14.5 mm LC, stade VIII), pour une même cohorte, dès la fin de sa première saison de croissance benthique. La transition de la vie planctonique à la vie benthique s'accompagne d'une chute du facteur de croissance au stade V, cependant compensée par la croissance élevée aux stades VI et VII. La combinaison du

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comportement cryptique, de la sélection pour les substrats rocheux, la déposition graduelle en fin d'été ainsi que la variabilité du facteur de croissance reflètent l'ajustement très fin des premiers stades de vie benthique du homard aux contraintes physiques de son milieu.

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Although the behaviour and habitat preferences of juvenile lobsters, *Homarus americanus*, have been studied in the laboratory, little is known of their early life stages in nature. Bottom selectivity appears as early as stage IV for *H. americanus* larvae, with the ability to delay their settlement if only unsuitable bottom types are available (Botero and Atema 1982; Cobb et al. 1983). When the bottom is not suitable the lobster will continue a succession of "touch-downs and liftoffs" until an appropriate substratum is encountered (J. S. Cobb, cited in Aiken and Waddy 1986). The positive correlation between stage IV larval densities and the proximity to shallow rocky bottoms further indicates that selectivity for bottom type starts during the planktonic stages (Hudon et al. 1986).

Benthic settlement occurs preferentially on loosely piled stones or shells (Pottle and Elner 1982) although U-shaped tunnels can be dug in a muddy bottom (Berril and Stewart 1973). Previous field records of juvenile lobsters report their occurrence exclusively on rocky bottoms (Bernstein and Campbell 1983; Elner and Hamet 1984; Cooper and Uzmann 1980; Scarratt 1973; Cobb 1971; Wilder 1953). However, most reports of natural densities are low (0.007–0.24 ind$\cdot$m^{-2}) and are associated with a mean size of over 40 mm carapace length (CL). Areas where high density (3.25 ind$\cdot$m^{-2}) (Cooper and Uzmann 1980) and small mean size (approximately 20 mm CL) (Bernstein and Campbell 1983) are found simultaneously are yet to be described. Characterization of the areas in which postlarval and juvenile lobsters predominate would permit protection and enhancement of natural nursery areas, thus ensuring survival of the critical early life stages on which recruitment into the commercial fishery depends.

Laboratory and mass rearing experiments have shown that even at an early stage, lobster density influences survival and growth rates by increasing the frequency of social, agonistic, or cannibalistic encounters (Van Olst et al. 1975; Cobb and Tamm 1974). Variability in growth rate was found to be high in seminatural enclosures (Roach 1983) as well as in the laboratory (Hughes and Mathiessen 1962; Templeman 1948a). Preliminary tagging experiments of small lobsters (Bernstein and Campbell 1983) also support this, although no complete record of natural growth between stages V and XIV has been available prior to this study.

During the time period spanning the larval and adult phases, important behavioural changes have been reported. Lobsters <25 mm CL spend most of their life in their burrows (MacKay 1929) whereas they are increasingly often found walking outside as they reach 25–35 mm CL (Roach 1983). The change of behaviour occurs over the same size range as the allometric transition from predominance of the abdomen to that of the cephalothorax (Lang et al. 1977). On this basis, small lobsters often attempt to escape predators by abdomen flexing whereas large lobsters tend to defend themselves with their claws. It is therefore expected that small lobsters should become progressively more visible and active in the open as their increase in size makes them less vulnerable to predators.

We studied the ecology of the early benthic stages of lobster off Îles de la Madeleine, Quebec. Underwater observations while SCUBA diving were made to document the changes in behaviour occurring as individuals increased in size under natural conditions. Density, biomass, and size structure of lobsters captured on different bottom types were quantified. Three stations where large numbers of postlarvae were initially found were sampled at monthly intervals from May to October, which allowed evaluation of growth and of some sources of size variability. The data provide a general picture of the early benthic life stages of lobster and allow us to better understand the fine tuning of its life cycle with the physical environment.

Study Area

Îles de la Madeleine are an archipelago 100 km long located in the southern Gulf of St. Lawrence (Fig. 1). They constitute seven islands, five of which are linked by narrow sand dunes enclosing three lagoons. Sand is the most common substrate around the islands, covering 87% of the area (Table 1). The bedrock is soft red sandstone; rocky bottoms, where lobster fishing takes place, represent only 9.9% of the total surface within the 20-m isobath. Hard and soft bottoms are equally well represented on both sides of the Islands. The open lagoons, which represent only 3.1% of the surface available to lobsters, are shallow (<8 m) sandy basins supporting a heavy growth of eelgrass (*Zosteraa marina*) in the summer. Lagoons harbour transient adult male lobsters attracted inwards by the warm water temperature gradient in early spring (Munro and Therriault 1983).

The number of degree-days cumulated over the summer (June to September inclusive) varies markedly with depth. Shallow waters are the warmest, with little difference between the coast and the inside of the lagoons (Table 1). Deeper waters on the northwest side vary within a narrower thermal range than on the southeast side of the islands, presumably reflecting the tempering influence of the Gulf waters on the northwest side (Hudon et al. 1986). The two sides also exhibit different timing in the seasonal thermal regime, in which spring warming and fall cooling occur later on the northwest than on the southeast side (Fig. 2).

Materials and Methods

Field sampling was carried out from May 20 to September 27, 1985. Site exploration, substrate characterization, and sampling were conducted by SCUBA diving. Sampling sites were determined using information on larval distribution and the presence of hard bottom. Temporal fluctuations of lobster density were followed at the three coastal stations (Fig. 1). Two

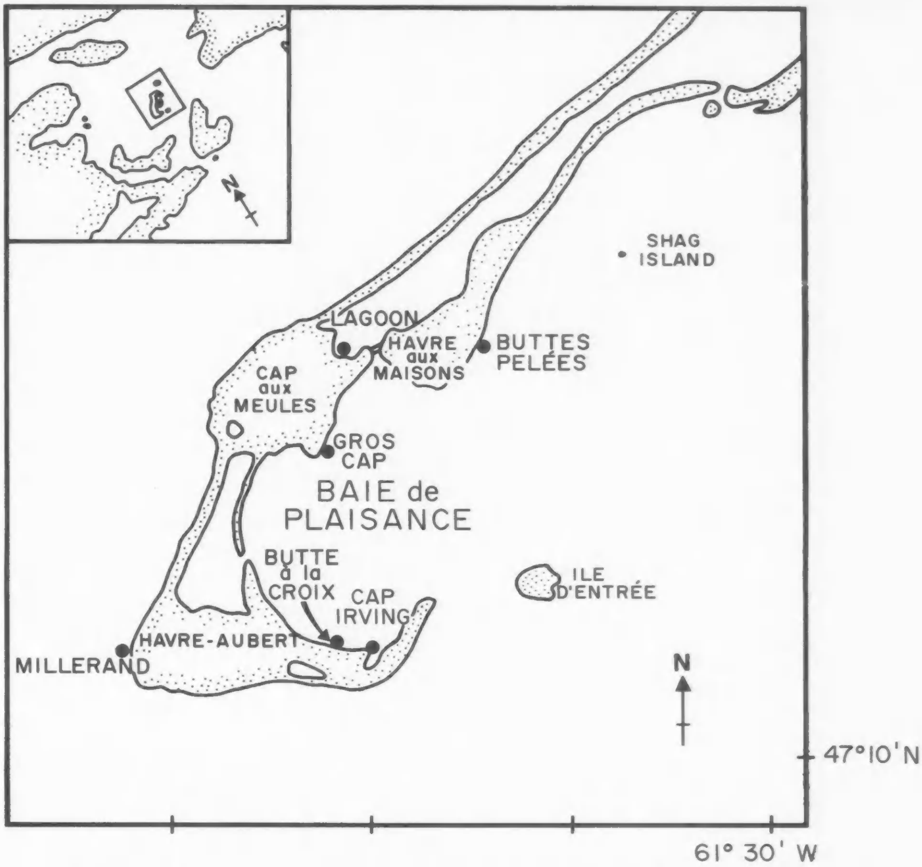

FIG. 1. Location of Îles de la Madeleine in the Gulf of St. Lawrence (inset) and of the sampling stations.

adjacent stations were located on the south side of Baie de Plaisance: Cap Irving and Butte à la Croix, commonly referred to together as Les Demoiselles. The third station was located on the north side of Baie de Plaisance (Gros Cap). Comparison of lobster density and size structure on different bottom types was made by sampling adjacent areas of rock and sand bottoms concurrently at each of these three stations. Additional bottom types were sampled in the Lagune du Havre aux Maisons, in Millerand and Buttes Pelées (Fig. 1).

Continuous temperature records were obtained throughout this period using Ryan model J thermographs immersed at Millerand (7 m), Gros Cap (8 m), Les Demoiselles (3 m), and Shag Island (7 m). Additional temperature readings at the surface and bottom were taken with each sampling.

Underwater Sampling

Quantitative sampling of lobster densities was carried out in daytime, using one of two techniques, depending on substrate type. On bare sand and eelgrass beds, two divers swam along a leaded line 100 m long, each diver observing 1 m on each side of the line. The eelgrass and the surface of the sand were raked for hidden or buried animals. This technique allowed examination of a large surface area (200 m^2) with minimal logistic preparation. This method was deemed appropriate on soft substrates, since lobster densities were low and their visibility was high.

On rocky bottoms, a seine net 2.5 m high (7-mm mesh size) was used to enclose an area 10 × 4 m. Escape of the smallest individuals was prevented by sewing a skirt of 3-mm mesh to the lower 0.45 m of the net and by adding lead to the bottom edge, which was manually fitted to the substrate. Escape over the top was unlikely, since sampling took place in shallow water (2–7 m) and the seine generally covered most of the water column.

The area suitable for the installation of a corral was selected by the availability of an area (10 × 4 m) with only one type of bottom material. Although this is not representative of the heterogeneity of rocky bottoms, where small patches of sand, gravel, and bedrock may be found within a few metres, it was deemed necessary to sample a bottom area as homogeneous as possible in order to allow comparison of lobster density and size structure on different bottom types. Areas previously sampled were avoided at stations where temporal changes were being monitored. Integrity of the sampling area was ensured by 0.4-m steel rods and guy ropes at each corner of the seine. Installation by two divers took 30 min to 1 h, including the preliminary search, corral positioning, driving in the rods, deploying the

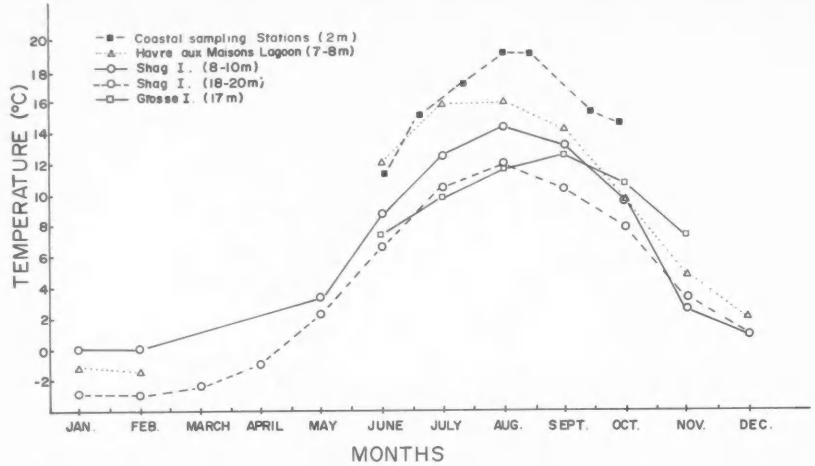

FIG. 2. Average monthly temperature recorded in different areas and depths off Îles de la Madeleine. Warmest waters are found over shallow rocky bottoms and in Lagune du Havre aux Maisons. Water warms more slowly on the northwest side (Grosse Île) than on the southeast side (Shag Island) at corresponding depths.

TABLE 1. Surface area of hard and soft bottoms found off the coast and in the lagoons of Îles de la Madeleine. The coastal area was divided into the southeast (SE) and northwest (NW) sides of the islands.

	Side	Surface (km^2 (%))[a]	Cumulated degree-days (>4°C·10^2)[b]
Coastal area			
Shallow rocky areas (≤5 m)	SE NW	3.69 (0.1) 6.98 (0.2)	13.6 ?
Deep rocky areas (5–20 m)	SE NW	176.36 (4.9) 168.10 (4.7)	6.8–10.0 7.4–9.0
Sand and mud (5–20 m)	SE NW	1625.64 (45.5) 1483.09 (41.5)	6.8–13.6 7.4–?
Lagoons			
Eelgrass beds (2–5 m)		64.57 (1.8)	11.8–13.4
Anoxic mud and dead eelgrass		44.80 (1.3)	11.8–13.4
Total		3573.23	

[a] Values digitized from maps of commercial fishing grounds prepared by MAPAQ (Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec) and aerial photographs.

[b] Values computed from Dobson et al. (1981) and Dobson and Petrie (1982, 1983, 1984, 1985) for the months of June to September inclusive.

net, fitting the lower edge, and installing the guys. The bottom was usually photographed for reference prior to sampling, using a Nikonos V™ underwater camera with a 15-mm lens.

Sampling in the corral was carried out by three divers moving side by side, each diver covering a corridor 1.3 × 10 m. All stones were overturned and crevices inspected for lobsters. The presence of other abundant species of animals was noted. Lobsters were captured manually, except for the smallest ones (5–10 mm CL), for which a wide-aperture syphon was occasionally used. Lobsters were placed in nylon mesh carrier bags filled with algae in order to minimize aggression between animals.

After allowing settlement of disturbed sediment, and rest for the sampling crew, the corral was sampled again by the same divers. This provided estimates of fishing success from successive captures using the same sampling effort.

Total lobster abundance (N) was estimated by the two-sample method assuming a constant sampling effort in an enclosed area. With two fishing dives, the following formula was used (Seber 1982):

$$(1) \quad N = \frac{m^2}{m - n}$$

in which m and n are the numbers of animals captured during the first and second dives, respectively. The variance (s^2_N) of the abundance is estimated by the formula of Seber and Le Cren (1967):

$$(2) \quad s^2_N = \frac{m^2 \times n^2 \times (m + n)}{(m - n)^4}.$$

This method of estimation of abundance can be used only if (1) $m > n$ and (2) $N \times p^3 > 16 \times q^2 \times (1 + q)$, in which q, the catchability coefficient, is estimated by n/m and p, the probability of capture, equals $1 - q$. On one occasion, the catch from the second dive was not much smaller than that of the first so that a third dive was then undertaken. In this instance, the abundance was calculated using the adaptation of the above formulae to three cases (Junge and Libosvarsky 1965).

Following the dives, the lobsters were sorted aboard the support vessel and maintained live under wet kelp. Carapace length (from the posterior edge of the eye socket to the posterior margin of the carapace, following a line parallel to the medio-dorsal suture) was measured to the nearest 0.1 mm with vernier calipers. Sex and missing appendages were noted. Pleopods were collected for moult stage determination (Aiken 1973). Lobsters were released at the Gros Cap station on the evening of capture, after having a uropod clipped or a plastic tag inserted into their abdomen. Releases were concentrated in a small area (approximately 20 × 20 m) in an attempt to increase the chances of recapture.

Some individuals were retained for wet weight measurements to the nearest 0.01 g. Biomass was calculated using the following length–weight relationship, calculated for both males and females, since no significant difference was found between sexes before sexual maturity:

(3) $\log_{10} W = -3.172 + 3.048 \log_{10} \mathrm{CL}, r = 0.99, n = 199$

in which carapace length (CL) is in millimetres and fresh weight (W) is in grams. The regression equation was based on 199 lobsters of size between 6 and 60 mm, excluding animals with missing appendages.

Estimation of Growth

Moult groups between stages V and XIV were estimated using MacDonald's (1980) program of analysis of distribution mixtures for size–frequency data, carried out on the 1-mm size interval frequency of cumulated catches. Initial values of mean size at each stage were determined by a visual examination of the modes and fitted by successive iterative calculation of proportion, mean size, and standard deviation values for each mode. The mean size at successive moult stages best fitting the observed values was determined by the lowest χ^2 value, indicating the absence of difference between the model and the cumulated size frequencies.

The growth factors (percent increment at moult) for each mean size at successive stages thus calculated were then compared with the growth factor values expected from Mauchline's (1977) equation:

(4) $\log_{10} \text{growth factor (\%)} = -0.005\mathrm{CL} + 1.517, \quad r = 0.99, n = 15.$

Growth factor values originally used by Mauchline (1977) to calculate equation 4 were taken from a study of Templeman (1948a), who interpolated growth factor values between stages IV and XIV. The initial value of carapace length used to initiate calculation in equation 4 is 4.83 mm CL, which is the overall mean size of stage IV larvae collected off Îles de la Madeleine (Hudon and Fradette 1985b).

The duration of the intermoult at each stage was calculated using a second equation from Mauchline's (1977) study:

(5) $\log_{10} \text{intermoult period (days)} = 0.013\mathrm{CL} + 1.129, \quad r = 0.93, n = 9.$

Equation 5 was derived from data on nine male lobsters between 4 and 35 mm CL (Templeman 1948a). In the present study, the moult stage and size attained by the end of the first benthic growth season (December 31) was estimated by adding the expected duration of intermoult, calculated from equation 5, for each successive stage until it equaled the length of the growth period remaining after the peak of settlement (70 d). The same calculation was used for subsequent years of growth, for which the total number of growing days was evaluated at 130 d.

Results

Ontogenetic Changes in Lobster Behaviour

The manual capture of over 1400 lobsters in SCUBA diving provided the opportunity to observe characteristic behavioural traits and to attempt to generalize the pattern associated with increasing sizes. Lobsters <20 mm CL were mostly found hidden under stones, except when disturbed by divers' activities. Once in the open, they appear to have a weak perception of divers' presence, as well as a low swimming ability. When confronted, they moved backwards in a succession of short bursts of tail flipping but could be easily captured by hand. These small lobsters thus appeared highly vulnerable to predation.

Lobsters between 20 and 30 mm CL were the most difficult to capture and were only occasionally seen in the open. They could detect the approaching hand of a diver and swam away swiftly to shelter, sometimes somersaulting and following an erratic course. One way to catch them was to provide a dark shelter by cupping one hand on the bottom and then making threatening movements towards the lobster with the second hand. The lobster would often respond by swimming directly into the cupped hand, possibly lured by the appearance of a dark shelter.

Lobsters >30 mm CL were commonly observed walking outside their burrows in daytime. They also showed good swimming ability using abdomen flexing. However, they were easily captured, since they first adopted the meral spread posture before fleeing. These observations suggest that a change in behaviour occurs with increase in size, reflected by the decrease of cryptic behaviour, the improvement of swimming ability, and the appearance, in the larger lobsters, of a defense reflex.

Following these observations, we designated lobsters according to their size and activity level. In the strict sense, "larvae" comprise the first three planktonic stages. As they reach stage IV, lobsters undergo a metamorphosis and enter the "postlarval" (decapodid) stage, during which the transition from planktonic to benthic life occurs (Felder et al. 1985). We considered that the postlarval stage extends through the early benthic life up to a size of about 25 mm CL, which is the middle of the interval over which we observed the behavioural change. "Juvenile" designates immature lobsters >25 mm CL. The "adult" stage is reached at sexual maturity, which approximates the minimum commercial size of 76.2 mm CL (Dubé and Grondin 1985).

Lobster Sizes on Different Bottom Types

Bottom type was characterized following Cooper and Uzmann (1980) because of the similarity of the nearshore macrofauna and flora in their study with that found in Îles de la Madeleine. The size structure of the lobster population varied widely according to different bottom types and the occurrence of macrophytes (Fig. 3). The bottom type on which the smallest mean sizes were observed (24.9 and 23.8 mm CL at Butte à la Croix and Cap Irving, respectively) was typically composed of small stones imbedded in sand with little or no algal cover (Table 2). This habitat provided only small crevices and little surface cover. No commercial-size lobsters were found on this bottom type, which although supporting high densities of small lobsters, had low total biomass. Postlarval and juvenile lobsters were mostly found under single rocks not totally embedded in the sediments. In many cases, the presence of the animal was betrayed by its chelae or its antennae extruding from a crevice. It was not possible to determine the extent of tunnel or burrow construction. This type of bottom was found at both Les Demoiselles stations (Cap Irving and Butte à la Croix) (Table 2).

In areas of larger stone size and heavier algal cover, lobsters were increasingly variable in size. In such areas, postlarvae, juveniles, and adults coexisted in high densities (1.20–3.80 $\mathrm{ind \cdot m^{-2}}$), as crevices of all sizes and shapes were abundant and

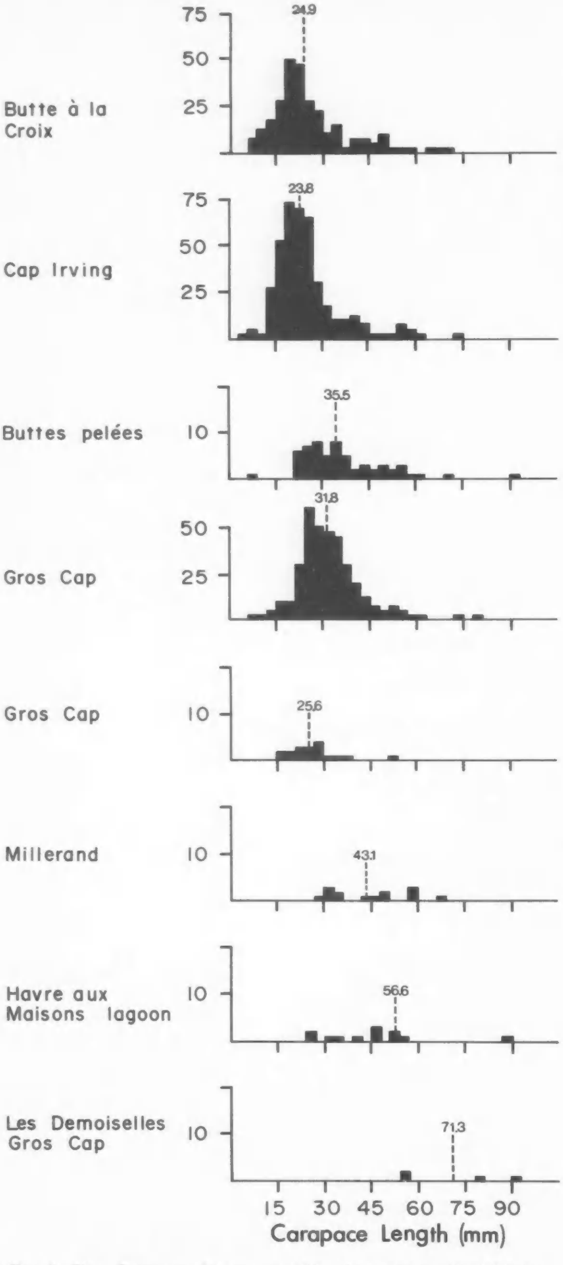

FIG. 3. Size–frequency histograms (CL, 3-mm interval) of lobsters captured on different types of bottoms. Vertical order of the histograms corresponds to that of the stations described in Table 2.

macroalgae provided additional surface cover. The impact of surface cover was particularly noticeable at Buttes Pelées (Fig. 3) where commercial size lobsters were found under algae. Accordingly, a high biomass was present on those bottom types, in spite of the small mean size (35.5 mm CL) (Table 2).

Sandstone substrate supported intermediate lobster densities (0.45–0.54 ind$\cdot$m^{-2}) (Table 2). Sandstone bedrock was pitted with holes 10–60 mm diameter bored by the bivalve *Zirphaea crispata* or the green sea urchin (*Strongylocentrotus droebachiensis*). Although the holes increased the complexity of this habitat, small lobsters were seldom found in the holes and were mostly hidden under stones. Accordingly, mean size and biomass appeared directly related to the amount of cover available under stones scattered on the bedrock (Table 2; Fig. 3). Lobsters >50 mm CL were observed when algae, piles of stones, or large crevices were present, such as in Millerand. Small mean size and low biomass were found on bare pitted sandstone off Gros Cap (Table 2).

Compacted sand and mud consolidated by eelgrass and *Ulothrix* sp. were predominant in the lagoons, which supported low densities (0.04 ind$\cdot$m^{-2}) of relatively large lobsters (mean size 56.6 mm CL) (Fig. 3). Lobsters were commonly found walking in the eelgrass or hiding under a mat of dead eelgrass. Evidence for tunnel digging was found for adult lobsters only.

Bare sandy bottoms appeared to be a poor habitat for lobster, as the lowest densities were recorded on this bottom type (Table 2). Lobsters found on sand could have been migrating from one rocky area to another. Only large lobsters (56.1–92.3 mm CL) were captured on the transects, although lobsters 30–40 mm CL were also observed off the transect or during exploratory dives. Wide bowl-shape depressions, possibly dug by commercial-sized lobsters as temporary shelters, were occasionally seen.

Temporal Fluctuations of Lobsters

Lobsters were sampled quantitatively throughout the summer at three sites characterized by rocky bottoms on which small lobsters were initially found. Density, biomass, and size structure were monitored throughout the summer at Les Demoiselles (Butte à la Croix and Cap Irving) and Gros Cap (Fig. 4). Comparison of the size structures shows similar trends at the three sites for different size groups. Lobsters <9 mm CL were observed in May at Cap Irving, disappeared for the following 2 mo, and were observed again at the three stations in mid-August (Fig. 4). The occurrence of such small individuals in May 1985 could be explained by larvae having settled in the late fall of 1984 and undergone little or no growth over the winter. Growth of these lobsters could have resumed as soon as spring warming occurred, which would explain their disappearance from May until the settlement, in August, of the lobsters hatched in the summer of 1985. It is remarkable that lobsters in the 9–12 mm CL class were seldom observed, so that lobsters <9 mm CL frequently appeared as an isolated mode.

Lobsters 15–30 mm CL formed a large mode which was found throughout the summer at all three stations (Fig. 4). The mode did not conspicuously progress to larger size classes with time as would be expected from the growth of a single cohort. Such a "stationary" mode could be produced by the simultaneous effects of differential growth, mortality, and emigration rates occurring over this size range, inducing an "accumulation" of individuals. Finally, the abundance of lobsters >30 mm CL fluctuated at all three stations, suggesting migrations closer to shore or towards deeper waters at different times during the summer.

Between June 8 and September 22, 559 marked lobsters captured at Les Demoiselles were released at Gros Cap, thus potentially increasing the density by 1.4 ind$\cdot$m^{-2}. Additionnally, 319 marked lobsters originating from Gros Cap were released at the site after handling. Half of the releases were

TABLE 2. Comparison of biomass and density of lobsters captured on different bottom types. The numbers of samplings (*S*) and of lobsters (*L*, in parentheses) captured are indicated for each station. The vertical order of the stations corresponds to that of the histograms shown in Fig. 3.

Site and depth	Bottom type (from Cooper and Uzmann 1980)	Macrophytes	S (L)	Density range (ind·m^{-2})	Biomass range (g·m^{-2})
Butte à la croix (2.5 m)	Sand base with stones	Absent	5 (314)	1.19–2.50	19.9–40.0
Cap Irving (2.5 m)	Sand base with stones	Absent	6 (453)	0.75–3.30	9.2–58.3
Buttes Pelées (4 m)	Sand base with stones	Macroalgae	1 (59)	1.80	82.3
Gros Cap (2–5 m)	Sand base with boulders	Macroalgae	4 (553)	1.20–3.80	42.7–119.7
Gros Cap (7.5 m)	Pitted sandstone (bedrock base)	Absent	1 (18)	0.45	8.6
Millerand (7 m)	Pitted sandstone with stones	Absent	1 (14)	0.54	48.3
Lagune du Havre aux Maisons (2–5 m)	Sand	Eelgrass	4 (15)	0–0.04	0–8.2
Les Demoiselles Gros Cap (2–5 m)	Sand	Absent	7 (4)	0–0.02	0–6.2

made by divers, while the other releases were from the surface. Releases by divers provided protection against the heavy predation by cunners (*Tautogolabrus* sp.) which was observed during surface releases. Of these 878 marked lobsters, only 16 were recovered, 9 with a tag and 7 with a clipped pleopod. All tag recoveries occurred shortly after the date of release, suggesting that tags were lost with time. Similarly, lobsters with clipped uropods were likely to regenerate the appendage within one or two moults, and thus were not recognizable after a few weeks. In summary, the manipulations at Gros Cap resulted in opposing effects on density. On one hand, natural density was increased because of releases of individuals from other sites. On the other hand, lobsters of both local and imported origins suffered a high mortality at release, especially when releases were made from the surface. For the sake of further comparisons, the maximum possible increase in lobster density on the Gros Cap site was evaluated at 0.7 ind·m^{-2}, assuming a minimal value of 50% mortality at release.

The Gros Cap results are nevertheless presented, since they demonstrate the high support capacity of this environment. It is noteworthy that Gros Cap densities, observed in July before the releases took place, were already 50% higher than observed simultaneously at Les Demoiselles. Sampling reliability was estimated at Gros Cap on two successive days in early August (Fig. 4). Density and biomass estimates were virtually identical, as both size–frequency histograms feature a mode in the 30–33 mm CL size class and another in a smaller class. By mid-August, density and biomass increased dramatically, due to the presence of a large number of lobsters 30–60 mm CL. Such an abrupt rise of 2.4 ind·m^{-2} is mostly of natural origin, since total releases made by that date could account for an increase of only 0.6 ind·m^{-2}. Even when releases are taken into account, lobster density and biomass were consistently higher in Gros Cap, among boulders covered with macroalgae, than in Les Demoiselles, among bare stones. Furthermore, lobster density and biomass were less stable in Gros Cap than in Les Demoiselles, possibly because of the presence, in Gros Cap, of larger lobsters migrating periodically to shore.

The above possibility can be examined if, in our consideration of seasonal variations of density, we take into account the movement capacity of each size group of lobsters. Following our behavioural observations and the findings of previous studies, the lobsters captured at each corral were divided into postlarvae (≤25 mm CL) and larger (>25 mm CL) lobsters (Fig. 5). Using this criterion, the variations of postlarval density were virtually synchronous at the three stations (Fig. 5). From a high in early June, densities steadily decreased until mid-August. A rise in postlarval density was then observed at all three stations when the first newly settled larvae were observed. The consistency of the trend shown for the three stations indicates that the number and proportion of postlarvae were not strongly modified at Gros Cap due to the releases.

For lobsters >25 mm CL, densities follow parallel fluctuations at Cap Irving and Butte à la Croix, always remaining below 1 ind·m^{-2} (Fig. 5). At Gros Cap, where larger lobsters were observed, densities were consistently higher (0.8–2.6 ind·m^{-2}) and less stable than at the other stations. In summary, postlarval lobsters are found in comparable densities on rocky bottoms with or without algal cover, but boulders covered with macroalgae can support a wider range of sizes and a higher biomass per square metre than bare stones.

In all samples, the female to male ratio ranged from 0.59 to 2.60 ($\bar{x}$ = 0.88). The proportion of individuals missing appendages ranged from 18.6 to 55.6% ($\bar{x}$ = 34.9%). The value is probably underestimated, since we assumed that individuals with freshly cut claws had been maimed during the sampling process and these were not considered for this statistic. For both measurements, no pattern was observed with time or station.

Growth Estimations

Pleopods were examined to determine the moult stage of all

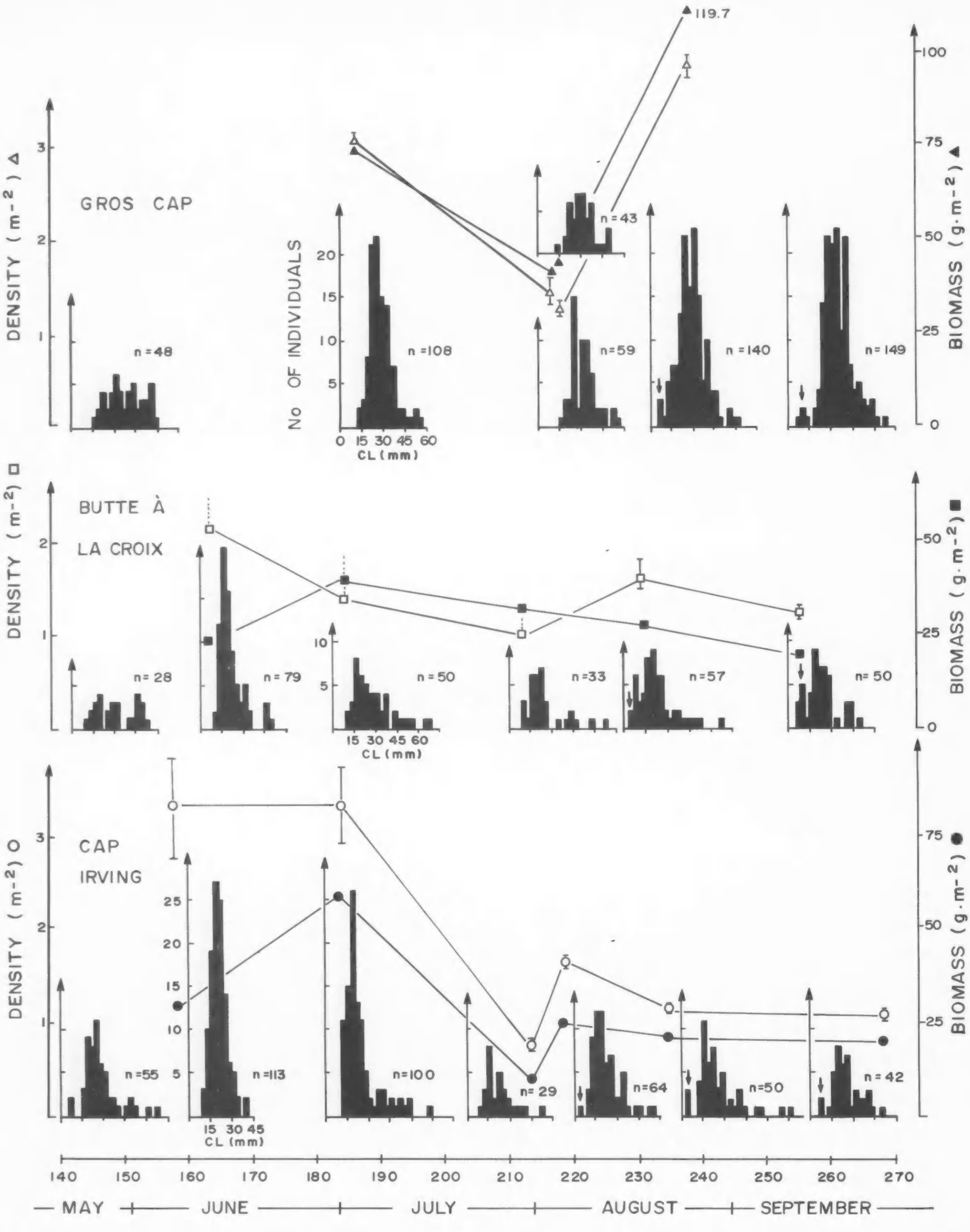

FIG. 4. Size–frequency histogram (CL, 3-mm interval) of all lobsters captured at the three shallow rocky stations at different times during summer 1985. All histograms are at the same scale. Vertical arrows indicate the occurrence of newly settled individuals.

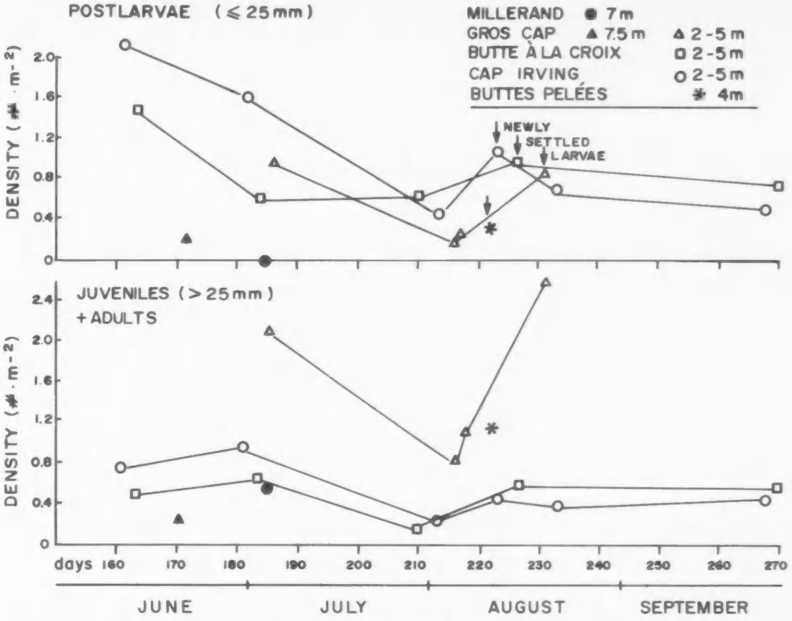

FIG. 5. Temporal fluctuations of density of postlarval (≤25 mm CL) and larger lobsters (>25 mm CL).

lobsters captured. Detailed analysis of moult stages showed that moulting took place throughout the summer at the three stations. This reflected the predominance of small lobsters, for which the intermoult period is short and increases progressively with size. The overall moulting activity was thus quantified as the percentage of lobsters in "active" premoult or postmoult condition, calculated on the number of lobsters in all moult stages, excluding C_4 (intermoult) and D_0 ("passive" premoult) stages.

The general pattern of seasonal moulting activity was similar for both size groups of lobsters (Fig. 6). No moulting activity was observed before the middle of June. In early July, a peak of moulting occurred simultaneously at all three stations, at which time 37% of the lobsters <25 mm and 30% of lobsters ≥25 mm were about to moult. After the July peak period, the average proportion of lobsters moulting equaled 14% for both size groups. At the beginning of August, low moulting activity was recorded for both groups, followed by a progressive rise in August and September. The increase in proportion of lobsters moulting was more apparent in the fall for lobsters ≥25 mm CL.

The size at successive moults was estimated by the examination of modes from size frequency histograms, both visually and by statistical (MacDonald and Pitcher 1979) methods. Since the number of moult stages is large and the size intervals between early stages are small, the statistical analysis was carried out on cumulated length frequencies of 1-mm interval between 4 and 53 mm CL (Fig. 7). The distribution fitted using MacDonald's (1980) program was not significantly different from the cumulated size frequencies ($\chi^2 = 51.53$, df = 39, $p = 0.085$) and showed the occurrence of 10 modes, corresponding to moult stages V–XIV. Expected modal values obtained from Mauchline's equation 4 were found to be significantly different from the cumulated size frequencies ($\chi^2 = 68.08$, df = 39, $p < 0.005$).

The first mode observed in the distribution, corresponding to a mean size of 5.30 mm CL, was allocated to stage V because of the occurrence of very small swimmerets on the first abdominal segment (Templeman 1948b). The standard deviation associated with each stage increased gradually from 0.43 at stage V to 3.99 at stage XIII, indicating a rapid increase of the scattering of sizes with moult stage. The standard deviation of stage XIV is likely to be underestimated due to the interruption of the analysis of modes at 53 mm CL due to decreasing numbers of individuals in the larger size classes.

The sizes at each moult stage were then used to calculate the duration of the intermoult (Table 3) and to estimate the stage that newly settled larvae would reach by the end of their first growing season (70 d) (Fig. 8). A previous study of larval ecology in the same area showed that most stage IV larvae were captured in August (Hudon et al. 1986). This would result in a benthic deposition period extending from August to October, depending on the rate of water cooling in the fall (Caddy 1979). Following these estimations, the first-year cohort would span stages V and VI for the last settlers in the fall and stage X for the August settlers (Fig. 8). The modal stage attained by postlarvae having settled during the peak period should be stage VIII, corresponding to a mean size of 14.5 mm CL at age 1 (December 31). By the end of the second and third year of growth, most lobsters in the cohort should reach stage XII and XIV, respectively, considering that about 130 d are available for growth in a full season.

The analysis of successive modes obtained from the size–frequency distribution revealed no correlation between growth factor (percent increment at moult) and carapace length ($r = -0.2624$, $n = 13$, $p > 0.05$). This finding is contrary to the relation interpolated by Templeman (1948a), which was later developed into an equation by Mauchline (1977) ($r = 0.996$, $n = 15$, $p = 0.001$). However, when both sets of data were

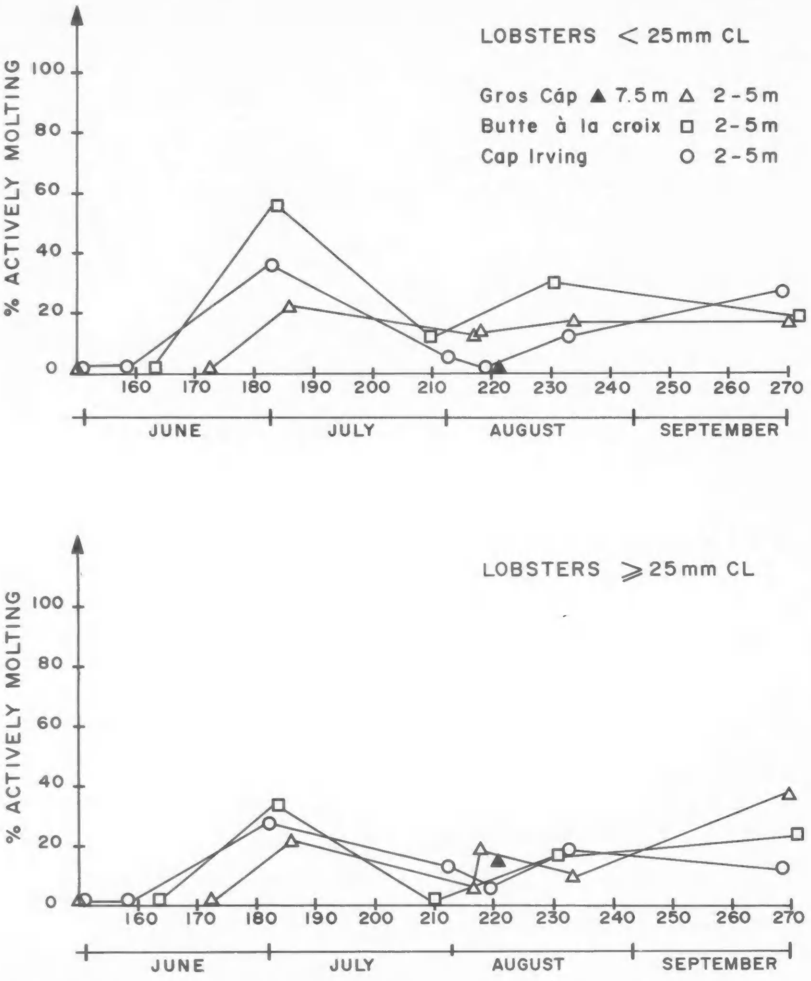

FIG. 6. Proportion of actively moulting lobsters (all stages except C_4 and D_0) for postlarvae (<25 mm CL) and larger lobsters (≥25 mm CL).

plotted together (Fig. 9), growth factor values obtained in this study showed the occurrence of periodical oscillations, dampening progressively on each side of the smooth line derived from Mauchline's (1977) equation. In spite of their different appearances, both relationships start at the same initial size value for stage IV (4.83 mm CL) and predict the size reached at stage XIV within 2 mm CL of each other. The first major discrepancy between the two relationships is observed at stage V, for which the percent increment at moult is at its lowest value (8.87%). High values are then observed for stages VI and VII, just before dropping again at stage VIII, which is the last stage most lobsters of the cohort should reach before their first winter (Fig. 8; Table 3). In their second growth season, growth increment at moult is high for the moults occurring in the early part of the summer and decreases at the approach of the second winter. The same pattern is repeated during the third growth season where the spring moult represents a larger percent increment than the fall moult.

Discussion

Impact of Habitat on Size Structure, Density, and Biomass of Lobsters

In Baie de Plaisance, annual production of planktonic stage IV larvae is in the order of 5800 ind·km^{-2} (Hudon and Fradette 1985a). Baie de Plaisance covers 240.47 km^2, 91.2% of which is sand, 7.6% is deep (>5 m) rocky bottom, and the remaining 1.2% is shallow (<5 m) rocky bottom. The number and density of postlarval lobsters produced should vary according to larval selectivity for, and availability of, the different bottom types. In the absence of a bottom selection process, but with survival restricted to the random settling of the larvae on rocky bottoms, densities of 0.006 ind·m^{-2} would be expected. If stage IV postlarvae settled preferentially on rocky bottoms, randomly distributed in relation to the depth–temperature gradient, densities of 0.07 ind·m^{-2} should be observed. However, if they

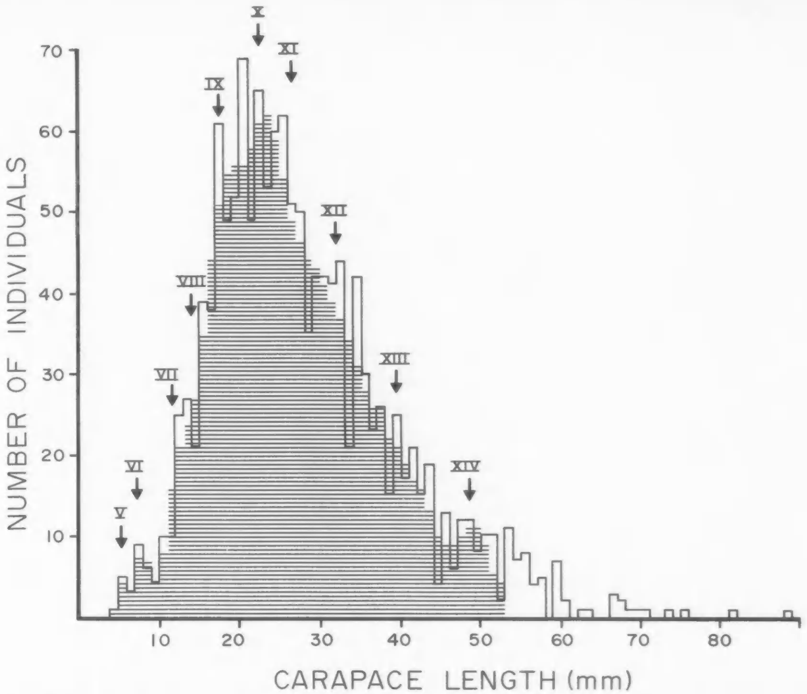

FIG. 7. Cumulative size–frequency histogram (CL, 1-mm interval) of all lobsters (n = 1412) captured on rocky bottoms. This histogram was used as the observed frequency distribution to which was adjusted a theoretical distribution (shaded area) between 4 and 53 mm CL. The model of mean values of size expected at stages V–XIV (vertical arrows) was adjusted using MacDonald's (1980) program.

TABLE 3. Mean size, standard deviation (SD), and proportion of each mode for successive moult stages calculated from a distribution adjusted to the cumulated size–frequency histogram. Growth factor represents the percentage of increment at moult from the preceding stage. Duration of intermoult was calculated using Mauchline's (1977) equation 5.

Moult stage	Mean carapace length (mm)	SD	Proportion of each mode (%)	Growth factor (%)	Duration of the intermoult (d)
I	1.99				
II	2.73			37.19	
III	3.60			31.87	
IV	4.83[a]			34.17	
V	5.30	0.43	0.44	8.87	15.8
VI	7.76	0.92	1.44	46.42	17.0
VII	12.00	1.32	3.45	54.56	19.3
VIII	14.50	1.84	2.98	20.83	20.8
					Age 1
IX	18.12	2.44	22.41	24.97	23.2
X	23.03	2.22	18.26	27.10	26.8
XI	27.24	2.88	16.09	18.28	30.4
XII	32.45	3.45	18.19	19.13	35.6
					Age 2
XIII	40.73	3.99	13.05	29.93	44.9
XIV	49.24	1.95	3.7	22.40	58.8
					Age 3

[a] Overall mean size values for stages I–IV obtained from Hudon and Fradette (1985b).

selected only the shallow rocky areas, densities of stage V postlarvae would reach 0.48 ind$\cdot$m^{-2}. Those figures drop to 0.004, 0.043, and 0.317$\cdot$m^{-2}, respectively, if a 30% natural mortality between stages IV and V, occurring at settlement time, is taken into account. In this study, densities of 0.18 stage V$\cdot$m^{-2} were observed on shallow rocky areas, which would support the hypothesis of an active selection of rocky substrata, with some preferential settlement, or better survival, in shallow waters. Also supporting this hypothesis is the significant negative correlation (r = −0.6675, p = 0.009) previously found between stage IV density and the distance to rocky bottoms in Baie de Plaisance (Hudon et al. 1986).

Once the postlarvae have settled, they hide under stones, probably to avoid predation. High densities of postlarvae were found on rocky substrata, irrespective of the presence or absence of algae. Juvenile and adult lobster densities were more closely related to the availability of large crevices among stones and/or of macrophytes providing surface cover, such as in Gros Cap, Buttes Pelées, and Lagune du Havre aux Maisons. Large adult lobsters, which may be immune to local predators during the intermoult period, were found on bare sand (Table 2; Fig. 3).

The relationship between substrate coarseness and size structure was previously demonstrated for European *(Homarus gammarus)* (Howard 1980) as well as American (Aiken 1980; Cobb 1971) lobsters. In addition, the high lobster density and biomass observed in Gros Cap indicates that this type of habitat has a high carrying capacity. For postlarval lobsters, density-dependent limitations, such as shortage of food or shelter, are less likely to occur in a complex environment such as a boulder

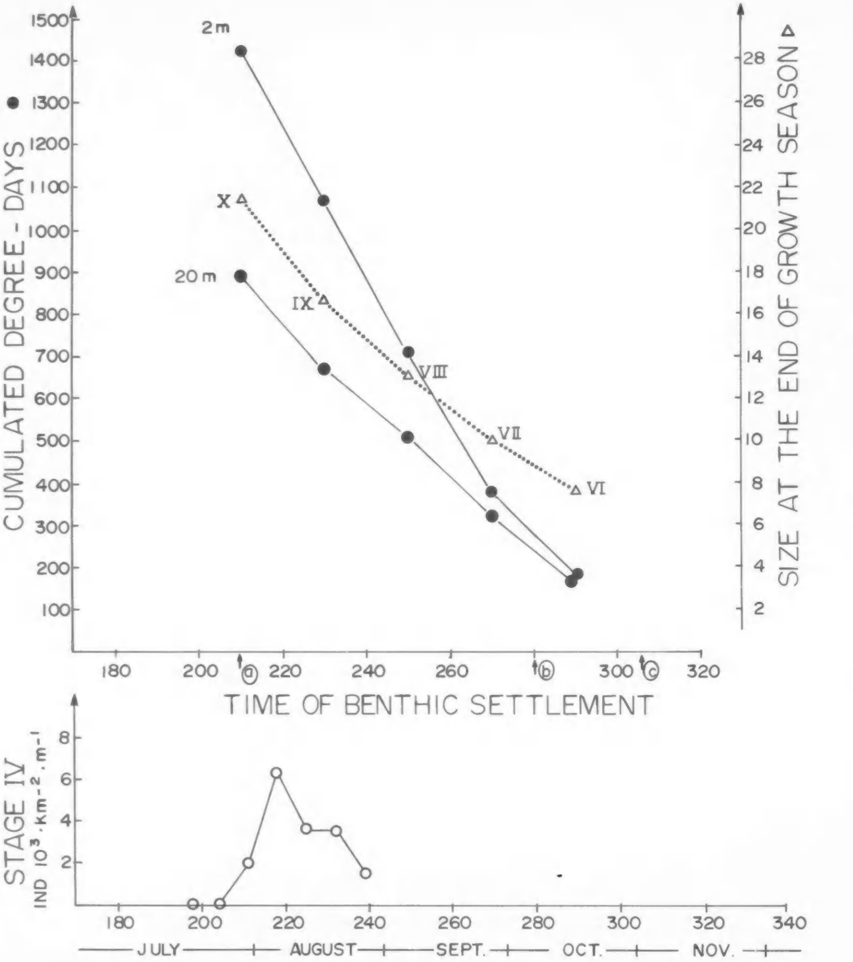

FIG. 8. Size attained by juvenile lobsters at the end of their first growing season in relation to the time of benthic settlement following the adjusted size values (Table 3; Fig. 7). The duration of the intermoult period was calculated using Mauchline's (1977) equation 5. The numbers of degree-days available for growth at 2 and 20 m depths are also shown to indicate range of physical conditions that postlarvae could encounter. The period of benthic settlement extends from the beginning of August (a) to early October (b), with the peak of settlement occurring during the last 2 wk of August. The end of the active growth season (c) is determined by the decrease of water temperature to 4°C.

field covered with macroalagae. Densities up to 62 postlarvae$\cdot$m^{-2} were found on rocky bottoms in a semi-enclosed basin experiment carried out with the European lobster (Bertran 1984).

Ecological Interactions between Growth Rate and Benthic Deposition

The overriding influence of temperature on lobster growth rate (Aiken 1980) should result in differential growth for larvae settling at different depths. Larvae settling in shallow waters, on which this study was concentrated, should benefit from a constantly higher number of degree-days during summer months (Table 1; Fig. 2). The lower temperatures in deep water are likely to result in an increase of the intermoult duration and an overall slower growth. Therefore, in deep waters, each moult stage should be reached later than in shallow waters. Calculation of the intermoult period using Mauchline's (1977) equation 5 does not account for temperature and assumes that high summer temperatures and low winter temperatures cancel out over the year. However, such conditions are only experienced by lobsters over 1 yr old, since postlarvae undergoing their first season of benthic growth are exposed only to decreasing water temperatures. A plot of the actual duration of intermoult, superimposed on the values expected from equation 5, would probably exhibit oscillations similar to those observed for the growth factor. Laboratory studies report that lobsters can reach stages IX–XII (Hughes and Mathiessen 1962) or VI–VIII (Wilder 1953) by the end of their first growth season. These values appear conservative when compared with the six-moult

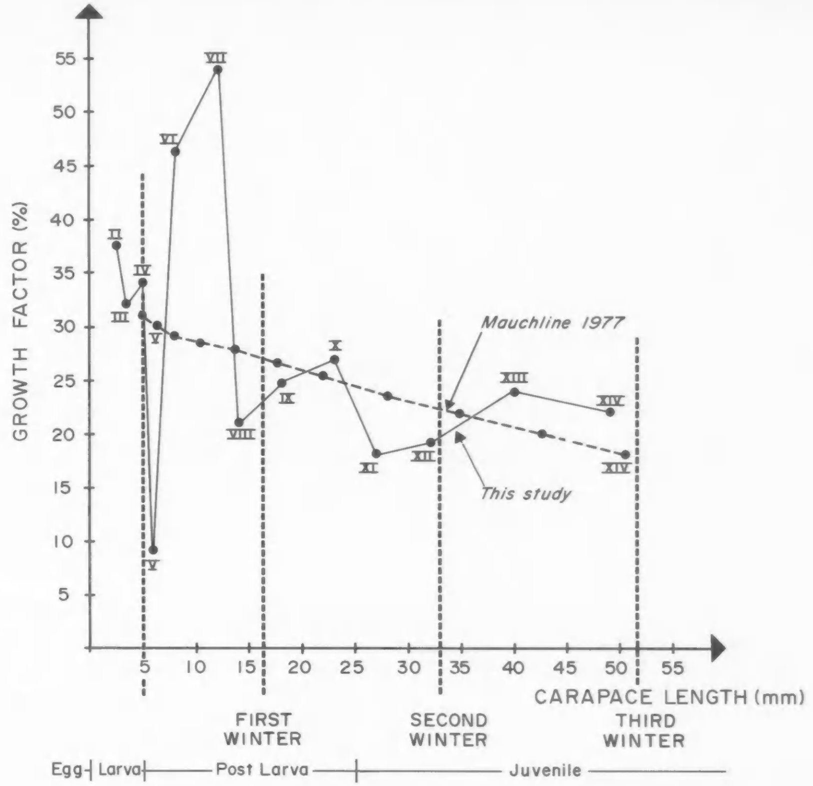

FIG. 9. Values of growth factor (% increment at moult) plotted as a function of lobster size for mean sizes calculated in this study and compared with the sizes predicted from Mauchline's (1977) equation 4.

span (stages V–X) calculated in the present study. The difference in degree-day accumulation with increasing depth (Fig. 8) is largest in the early part of the settlement period and diminishes as surface water cools during the fall, and is likely to result in a wider size span for larvae settled in shallow waters than in deep waters.

Deep waters off Îles de la Madeleine appear to be always colder than shallow waters (Fig. 2). However, in certain areas of the Atlantic coast such as southern Nova Scotia, the benefits of settling in shallow water could be counterbalanced in deeper water by warmer winter temperatures (Campbell 1986), which would allow a slower but uninterrupted growth. Off Îles de la Madeleine, growth could be interrupted during winter, but could be compensated for by a higher growth factor in spring and early summer moults (Fig. 9). For planktonic larval stages, the moults occurring in the early part of the summer were also associated with a higher growth factor than moults occurring in late fall (Hudon and Fradette 1985b). According to Aiken (1980), at a temperature of 5°C lobsters will progress slowly through the moult stages to D_0 (passive premoult) and stop, but will continue slowly through premoult and can complete ecdysis in temperatures as low as 0°C provided that moult stage D_1 (active premoult) has been reached before the temperature drops to 5–6°C. Following this, growth could continue slowly over the winter period, so that active moulting could resume after only a minimal spring warming. A similar phenomenon is observed in our results, as the percentage of actively moulting lobsters peaked in early July to 37 and 30% for postlarval and larger lobsters, respectively. For both size groups, the percentage of actively moulting lobsters stabilized at a lower level for the remainder of the summer (Fig. 6).

In shallow, warm waters, the majority of newly settled larvae would spend their first winter in stage VIII (14.5 mm CL). Stage VII lobsters settled in 1984 should grow to stage IX in early spring 1985 and be replaced in late fall of 1985 by the larvae settled during the summer. Since our sampling covered only a small part of the period of occurrence of stage VIII, the 14.5 mm CL mode was very poorly represented in the cumulated size–frequency histogram (2.98%) (Table 3). Monthly sampling also made it difficult to detect changes in size structure in the presence of a continuous inflow of small individuals moulting every fortnight. In the second season, four moults are possible, but the number of moults drops to two in the third season. The increase of moult activity in the fall observed for lobsters ≥25 mm CL (Fig. 6) could precede the appearance of two well-defined moulting peaks in spring and fall for adults (Robinson 1979). If later development proceeds as expected, lobsters of a given cohort could enter the fishery between age 4 and 6, depending on their time of settlement and individual growth rate. Further studies are needed to determine the occurrence and intensity of postlarval settlement in deep waters and to evaluate early growth rate under colder water conditions.

The proportions of individuals observed in their first, second, and third growth season were 8.31, 74.97, and 16.75%, respectively. The pattern in proportions is subject to numerous factors, including yearly variation of recruitment, the timing and location of the initial benthic deposition, migration of individuals, and the variation in growth rates within a cohort. In addition to the growth differences induced by the time and depth of benthic settlement, further variation is attributable to intrinsic differences in growth rate among individuals. Stage IV lobsters kept in underwater trays showed a range of sizes from 14 to 27 mm (total length) after only 42 d of immersion (Roach 1983). Similar findings were reported by many authors reviewed by Hadley (1906) and Wilder (1953). In this study, differences in the percentage of growth increment at moult could also be induced by the high proportion of maimed individuals ($\bar{x}$ = 35%), for which the regeneration of appendages should lower the increment in carapace length or modify intermoult duration.

The transition from pelagic to benthic life is associated with a very sharp drop in the growth factor (Fig. 9), suggesting that during stage IV, more energy might be allocated to the search for an appropriate substrate than to foraging for food. The sudden decrease in growth rate at settlement time is largely compensated for by high rates during the next two stages, which possibly reflect abundant food resources as well as sedentary habits during early benthic life. A second transition period occurs in the second year, when lobsters grow into juveniles over 25 mm CL. The change from cryptic and sedentary behaviour to a more visible, mobile type of life is an indication of a shift in the factors limiting the foraging area of lobsters 25–30 mm CL. Increase in size brings with it a lower vulnerability to predators, but also higher food requirements, which may together account for the higher activity and larger foraging area of juvenile lobsters. Venturing outside sheltered areas possibly induces a shift in mortality rate from postlarval to juvenile stage, which might be reflected by the decrease in the proportions of stages X and XI (Table 3).

The optimal strategy for postlarval lobsters, then, could be to settle as early as possible so as to reach the highest possible size before the first winter. This leads one to expect that postlarvae would concentrate in the warmest areas, if the appropriate bottom type were present. Accumulations of newly settled larvae should not occur, since they rapidly grow to a less vulnerable size, except possibly in late fall and winter. Postlarvae would hide until the juvenile stage was reached. Beyond 25 mm CL, substrate coarseness and the quantity and size of available cover would largely determine the carrying capacity of the habitat and influence the density, biomass, and size structure of lobsters found in an area. All these elements combined ensure maximal survival to the adult stage and reflect the very fine tuning of a lobster's early benthic life stages to the many constraints of its physical environment.

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Simulation Model of English Sole (*Parophrys vetulus*) Population Dynamics in Washington and Oregon Coastal Waters

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We constructed an empirically based simulation model of population dynamics for English sole (*Parophrys vetulus*) occupying waters off the Washington and Oregon coast. While the original purpose for developing this model was to provide a tool for analysis of management questions, we also found it useful in evaluating hypotheses about biological processes in the early life history of sole. By comparing the ability of alternative sets of model hypotheses to reflect historical variation in abundance of age 4 recruits, we found some combinations of hypothesized mechanisms to be better than others. Approximately 61% of the interannual variation in recruitment was accounted for by a model which included (1) the effect of ocean temperatures on spawning timing, egg hatching, and fish growth, (2) density-dependent larval mortality, (3) age-dependent mortality of fish younger than age 4 yr, and (4) density-dependent growth of age 1 fish. This model explained more variation in recruitment than previous models of this population and emphasized the importance of timing of spawning on the subsequent abundance of the cohort.

Nous avons élaboré un modèle de simulation empirique de la dynamique des populations de sole anglaise (*Parophrys vetulus*) qu'on retrouve au large des côtes des États de Washington et d'Oregon. Nous avons d'abord élaboré ce modèle pour l'analyse des questions de gestion, mais nous nous sommes aperçus qu'il était également utile pour évaluer les hypothèses concernant les processus biologiques au début de la vie de la sole. En comparant l'aptitude de différents modèles hypothétiques de refléter la variation historique de l'abondance des recrues de 4 ans, nous avons découvert que certaines combinaisons de mécanismes hypothétiques semblaient meilleures que d'autres. Environ 61 % de la variation interannuelle du recrutement s'explique par un modèle qui comprend 1) l'effet des températures de l'océan sur le moment du frai, l'éclosion des oeufs et la croissance des poissons, 2) la mortalité des larves en fonction de la densité, 3) la mortalité des poissons de moins de 4 ans en fonction de l'âge et 4) la croissance des poissons d'un an en fonction de la densité. Ce modèle explique davantage la variation du recrutement que les modèles précédents pour la même population et souligne l'importance du moment du frai sur l'abondance subséquente de la cohorte.

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Early life history processes in fish populations have long been a focus of research (e.g. Lasker and Sherman 1981). In this paper, we describe a simulation model of a population of English sole (*Parophrys vetulus*) and explicitly include in the model detailed representation of early life history events including age- and density-dependent processes as well as oceanographic influences. The population represented in the model occupies the portion of the Oregon and Washington coast designated area 3A by the Pacific Marine Fisheries Commission (PMFC) (45°46′N to 47°20′N).

This model enabled us to evaluate the importance of several alternative hypotheses regarding processes which cause interannual variability in recruitment of English sole. For other fish species, a variety of abiotic and biotic factors have been found important in influencing recruitment (Shepherd et al. 1984). For English sole, Ketchen (1956) and Hayman and Tyler (1980) found correlations between recruitment strength and environmental factors, although causal mechanisms were not directly known. Kruse (1983) explored several causal mechanisms hypothesized to influence English sole recruitment and found egg production, timing of spawning, and larval advection to be important.

Using the unique wealth of information available on the PMFC area 3A stock of English sole, we evaluated hypotheses about the early life history processes that were different from those examined by Kruse (1983); we included age-dependent mortality, density-dependent growth, and density-dependent mortality whereas he did not. We evaluated our hypotheses by constructing different versions of the model, each with its own set of assumptions. These models were started with the population age structure observed for 1964. They were then run with historical oceanographic conditions, and the time series of recruitments forecast by each version of the model was compared with historical data.

The ultimate goal for building this model was to provide a tool to quantify the probability of correctly estimating time trends of fish populations during monitoring or experimental fishing programs. To meet this goal, we needed a simulation model which realistically incorporated the effects of environmental variability on recruitment. In a separate paper (Peterman and Bradford 1987b), we use this model to explore how success at estimating true population trends is affected by the (a) length of the observation period, (b) fishing mortality applied during that period, (c) variance in environmental factors, and (d) imprecision and inaccuracy of stock assessment methods.

The next few sections describe the components of population dynamics of English sole, their representation in the model, and criteria for evaluating the ability of alternative sets of model hypotheses to reflect past observations. Throughout this paper, a "year" refers to the 12-mo period from August through July rather than a calendar year. English sole do not spawn during June and July (Kruse and Tyler 1983), and these months form a natural break in their annual life cycle.

Readers who do not wish to know the detailed assumptions of model components can refer to Table 2 for a concise description of different versions of the model and can then move to the Results section.

Methods

Model Components

A general flow chart of the simulation model is shown in Fig. 1, and the parameter values for the final version of the model are in Table 1. Model components are described below, along with methods of parameter estimation. In several sections, we describe two alternative assumptions about a particular model component. Each of these alternative assumptions was explored in different versions of the model, as outlined in Table 2, in order to evaluate various hypotheses about processes acting on English sole recruitment.

Spawning

Most English sole spawn during the winter months although some spawning occurs in other months as well (Kruse and Tyler 1983). Kruse and Tyler (1983) used a model driven by historical ocean bottom temperatures to estimate the monthly distribution of spawning for the years 1954–77. The ocean bottom temperatures used were calculated from the model of Kruse and Huyer (1983). Kruse and Tyler's (1983) estimates of spawning timing corresponded well with field observations, so in the present model, we used these same algorithms and temperatures to calculate the temporal distribution of spawning within each simulated year (see Kruse 1983 for details).

Egg production and hatching

We calculated total annual egg production as the product of the female fish abundance, proportion of mature fish, and fecundity of each age, summed over all ages. Fecundity and proportion mature were calculated as functions of length, using relationships from Kruse (1983) and Harry (1959), respectively. Harry's maturity ogive gives 50% of the females mature at 30.7 cm length, which falls between the average sizes of 4- and 5-yr-old fish. The fecundity equation was $F = 0.0004558L^{3.6097}$, where F is number of eggs per female and L is length (millimetres). Mean length-at-age was calculated by the growth section of the model, described below. The number of eggs spawned each month was the product of total annual egg production by the total female population and the proportion of the annual spawning which occurred in each month, as calculated by the temperature-dependent routines described above. The number of eggs produced each month was multiplied by a sex ratio of 0.5 because the rest of the model only deals with female sole, a convention adopted by Hayman et al. (1980) because 95% of the catch is female.

Calculation of monthly egg hatching rates followed the model of Kruse (1983). We used the dome-shaped temperature-dependent hatching success relationship of Alderdice and Forrester (1968) for a salinity of 32 ppt. Because English sole eggs float to the surface, our simulated hatch success was calculated from monthly sea surface temperatures. These surface temperatures were monthly averages calculated for the Oregon coastal region (44–46°N, 124–125°W) by Kruse (1983).

Larval mortality

The sequence of events from spawning to age 1 is shown in Fig. 2. Larvae at age 50–70 d begin to metamorphose into juveniles and to switch from a pelagic to a demersal habitat (Laroche et al. 1982). In the model, we used a total of 2 mo for the pelagic larval period. Two types of larval mortality, density independent and density dependent, were tested in the model. In the density-independent case, a constant instantaneous monthly mortality rate of 1.209 was applied during the 2-mo larval period, based on our analysis of 1971 ichthyoplankton data (Richardson 1977; Richardson and Pearcy 1977).

For the density-dependent case, larval mortality was assumed to be a function of larval density, as rationalized by Cushing (1983) and Shepherd and Cushing (1980). In this density-dependent version, the instantaneous monthly mortality rate, M_L, that was applied to each monthly cohort of larvae was a linear function of the abundance of that cohort, N, following Beverton and Holt (1957):

$$(1) \quad M_L = a + bN.$$

The parameters of this density-dependent equation were fitted by trial, with the objective of obtaining the historical age 4 abundances, estimated from cohort analysis, as closely as possible (see Model Testing section). The values of the a and b parameters (Table 1) in the final version of the model gave the best fit out of eight sets of density-dependent parameters tested (parameter a ranged from 1 to 1.14 and b from 3.4×10^{-13} to 8.4×10^{-13}).

Juvenile and age 0–3 mortality

The duration of the juvenile period for each monthly cohort of fish, from metamorphosis to inclusion into the age 0 group the following year, ranges from 1 to 10 mo, depending on the month in which the cohort was spawned. For example, eggs laid in September would be larvae until November and juveniles until the following August 1, when they would become age 0 fish (0 indicating that no annulus was formed yet). We examined two hypotheses regarding mortality during the juvenile stage.

The first hypothesis asked whether spawning date and therefore length of the juvenile period affects survival of the cohort. We tested this hypothesis by comparing Versions 1 and 2 of the model (Table 2). Version 1 assumed that all eggs were laid in December and that fish were therefore 6 mo in the juvenile stage before they entered the 0 group. Juvenile

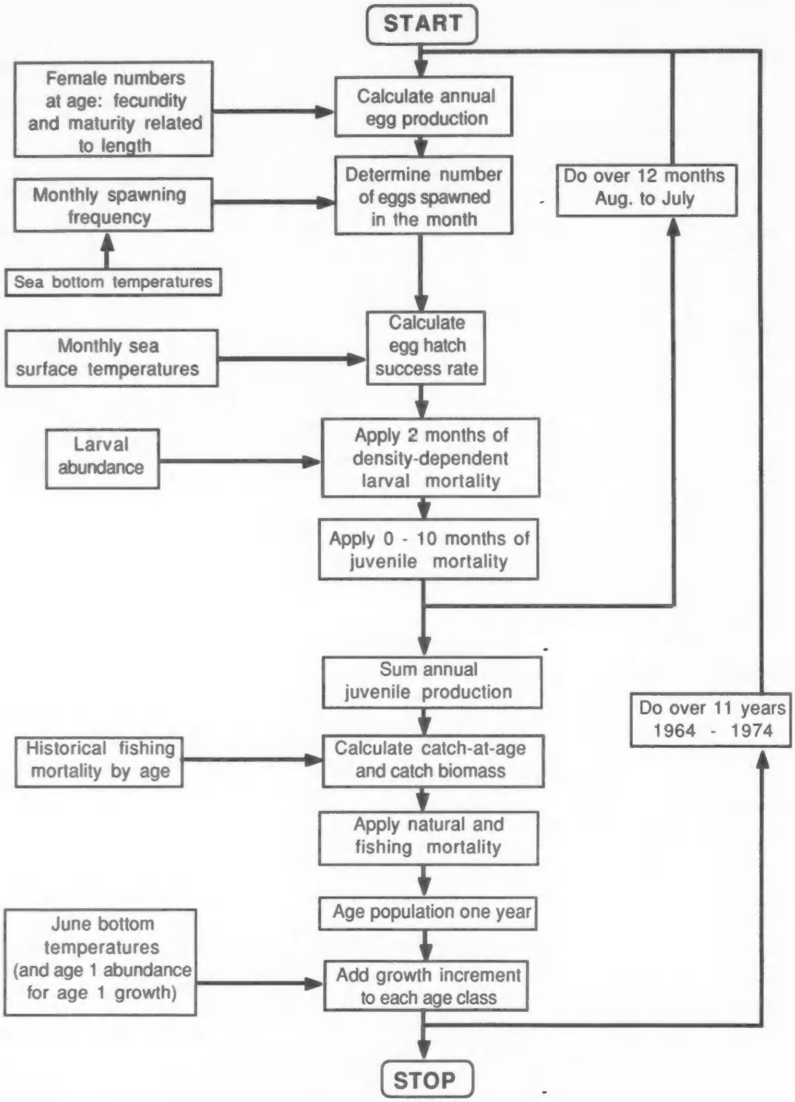
START
Female numbers at age: fecundity and maturity related to length
Calculate annual egg production
Monthly spawning frequency
Determine number of eggs spawned in the month
Do over 12 months Aug. to July
Sea bottom temperatures
Monthly sea surface temperatures
Calculate egg hatch success rate
Larval abundance
Apply 2 months of density-dependent larval mortality
Apply 0 - 10 months of juvenile mortality
Sum annual juvenile production
Do over 11 years 1964 - 1974
Historical fishing mortality by age
Calculate catch-at-age and catch biomass
Apply natural and fishing mortality
Age population one year
June bottom temperatures (and age 1 abundance for age 1 growth)
Add growth increment to each age class
STOP

FIG. 1. Model flow chart.

mortality was applied at the rate calculated in the Appendix, Section A. In Version 2, duration in the juvenile phase depended on spawning date, and the monthly juvenile mortality rate (derived in Appendix 1, Section B, Part 1) was applied over the appropriate number of months. As a result, model fish spawned in October, for example, experienced higher total mortality before becoming age 0 in August than those spawned in April. If spawning date and length of the juvenile period are important, then Version 2 should account for a greater portion of the interannual variability in historical recruitment than Version 1.

The second hypothesis that we explored was that mortality rates are age dependent prior to maturity, as has been found by Cushing (1975) for plaice (*Pleuronectes platessa*). We examined this hypothesis by comparing several versions of the model (4, 7, and 9) which had mortality rates that decreased from juvenile to age 3, with otherwise identical versions (1, 6, and 8, respectively) which assumed constant mortality rates during these ages. Mortality rates for the age-dependent version are shown in Table 1, and rates for both age-dependent and age-independent versions are derived in the Appendix, Section B. Versions 6–9 included a variable length juvenile period because that turned out to be the best of the two assumptions which were described in the previous paragraph (see Results).

Adult mortality (natural and fishing)

We used the instantaneous annual natural mortality rate for adults, M_A, of 0.294 for ages 4–13, as estimated by Hayman et al. (1980). Age-specific fishing mortalities for each year for ages 4 and above were from the cohort analysis of the historical

TABLE 1. Baseline parameter values used in the final Version (9) of the English sole model.

Symbol	Value	Definition
a	1.05/mo	Intercept of density-dependent instantaneous monthly larval mortality function
b	6.4×10^{-13}	Slope of density-dependent larval mortality function
M_J	0.727/mo	Instantaneous monthly juvenile mortality rate
M_0	3.551/yr	Instantaneous annual mortality (age 0)
M_1	1.904/yr	Instantaneous annual mortality (age 1)
M_2	1.021/yr	Instantaneous annual mortality (age 2)
M_3	0.548/yr	Instantaneous annual mortality (age 3)
M_A	0.294/yr	Instantaneous annual adult mortality rate (ages 4–13)

catch-at-age data (Kruse 1983). Natural and fishing mortality rates, for each age, were used at the end of each model year in the Baranov catch equation (Ricker 1975) to calculate the total catch and survivors in each age class. The catch actually landed was the product of the catch by age given by the Baranov catch equation and the age-specific utilization rates (Hayman et al. 1980). These utilization rates reflected the tendency for fishermen to throw back undersized fish, and all of those thrown back were assumed to die because of the lengthy period between haul-out of the nets and sorting (Ellen Pikitch, Marine Science Center, Newport, OR, pers. comm.). We assumed that no fish lived beyond age 13. The biomass of the landed catch was calculated from the estimated length-at-age at that year, the numbers at age in the landed catch, and the length–weight relationship (derived from Oregon landings of English sole by Ellen Pikitch (pers. comm.)):

$$W = 1.47 \times 10^{-5} L^{2.916}$$

where W = weight (kilograms) and L = length (centimetres).

Growth

Annual growth in length of age 1 English sole has been negatively correlated with June sea bottom temperatures over the years 1961–74 (Kreuz et al. 1982). In our analysis of historical data, we also included an additional effect of abundance of age 1 fish on age 1 growth (Peterman and Bradford 1987a); temperature and abundance accounted for most of the annual variation in age 1 growth (equation on line 1 of Table 3; multiple $r^2 = 0.91$, $P = 0.0001$, $n = 11$).

We tested whether density-dependent age 1 growth had any effect on recruitment by comparing Versions 3 and 6, which included this effect, with Versions 1 and 2, which did not. In the models, the length of all fish which just became age 1 in August was set to 11.4 cm (Barss 1976) because no data exist on interannual variation in growth during the preceding juvenile period. When density-independent growth was assumed, the equation of Kreuz et al. (1982) was used to calculate the age 1 growth increment as a function of temperature only. In the case of density-dependent age 1 growth, the equation shown in Table 3 was used to calculate age 1 growth from temperature and cohort abundance.

For ages 2–7, the temperature-dependent growth equations (Table 3) calculated by Kreuz et al. (1982) were used (not all were reported in that paper). For consistency, the point estimates of the parameters of these equations were used in our model for all ages, even though the regressions for ages 4 and 6 were not statistically significant. The growth of age 8–13 fish was calculated from the growth of age 7 fish multiplied by an age-dependent scaling factor (Table 3). For all ages, June bottom temperatures, which were used to calculate growth in a given year of the simulation, were estimated by Kruse and Huyer's (1983) algorithm for each year.

TABLE 2. Assumptions of different versions of the English sole model (+ = present, − = absent). The first assumption (juvenile duration) accounted for the effect of monthly spawning timing on each monthly cohort's duration of the period between metamorphosis and age 0 (August 1). In contrast, Versions 1, 3, 4, and 5 assumed that all juveniles produced in a model year had the same 6 mo in the juvenile period. "Density-dependent growth" refers to the effect of age 1 abundance on the annual age 1 growth increment, when incorporated into the multiple regression of growth on bottom temperature and abundance. When this assumption was not made, age 1 growth was only temperature dependent. "Mortality rate decreasing with age in juvenile–3" refers to the exponential decay relation assumed for the metamorphosis-through-age 3 period. In the absence of this assumption, the same annual mortality rate of 2.216 was used for each age. "Density-dependent larval mortality" assumes a linear relation between M_L and larval abundance. When this assumption was not used, M_L was set to 1.209.

Version	Juvenile duration	Density-dependent growth	Mortality rate decreasing with age in juvenile–3	Density-dependent larval mortality
1	−	−	−	−
2	+	−	−	−
3	−	+	−	−
4	−	−	+	−
5	−	−	−	+
6	+	+	−	−
7	+	+	+	−
8	+	+	−	+
9	+	+	+	+

Model Testing

In the sections above describing the model, we made several alternative sets of assumptions on some of the components because of limited data (e.g. density-dependent larval mortality, age-specific mortality in ages 0–3, etc.). Therefore, we tested several distinct and increasingly complex versions of the model (Table 2) to find the set of assumptions which best reflected the field situation, as described by the time series of historical age 4 abundances (from cohort analysis of Kruse 1983).

Starting conditions

During these tests, we drove the model with the historical sequence of age-specific fishing mortalities (Kruse 1983), spawning timings (Kruse and Tyler 1983), and oceanographic conditions (Kruse and Huyer 1983; Kruse 1983), beginning in 1964 and extending for 11 yr. The starting populations for ages 4–9 were from the 1964 population as estimated by cohort analysis (Kruse 1983). The 1964 abundances of ages 10–13 (not available from his cohort analysis) were the average abundances in those ages which resulted from cohort analysis over all ages 4–13. The 1964 age 0–3 abundances were estimated by "hindcasting" from age 4 estimates, dividing by the survival rates of the model version being tested. We then simulated the

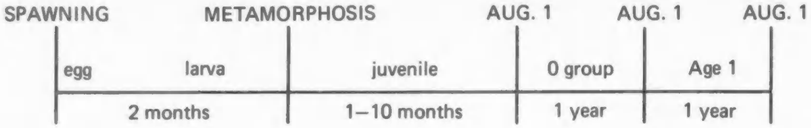

FIG. 2. Sequence of events in the early life history of English sole, as represented in the model. Times on bottom part of scale represent durations of the periods.

TABLE 3. Parameters of regressions describing Y, annual English sole growth increment (cm), as a function of X_1, June bottom temperature (°C), and for age 1 only, X_2, cohort abundance as indexed by thousands of age 4 fish 3 yr later: $Y = b_0 + b_1X_1 + b_2X_2$. n = sample size; r = correlation coefficient; $*P < 0.05$; $**P < 0.01$. Coefficients of age 8–12 equations were calculated as the product of the age 7 coefficients and the ratio of the average growth increment of a given age and the age 7 increment determined from a von Bertalanffy equation fitted to the length-at-age data for the Astoria population, from Kreuz (1978): $L_t = 41.9(1 - e^{-0.286(t+0.864)})$.

Age (yr)	n (yr)	r	b_0	b_1	b_2
1	11	−0.953**	18.94	−1.50	-1.37×10^{-4}
2	11	−0.827**	16.40	−1.35	—
3	11	−0.711*	8.82	−0.57	—
4	11	−0.487	5.32	−0.28	—
5	10	−0.743**	5.43	−0.39	—
6	9	−0.178	2.59	−0.08	—
7	8	−0.680*	3.19	−0.20	—
8	—	—	2.04	−0.13	—
9	—	—	1.53	−0.10	—
10	—	—	1.15	−0.07	—
11	—	—	0.89	−0.06	—
12	—	—	0.67	−0.04	—

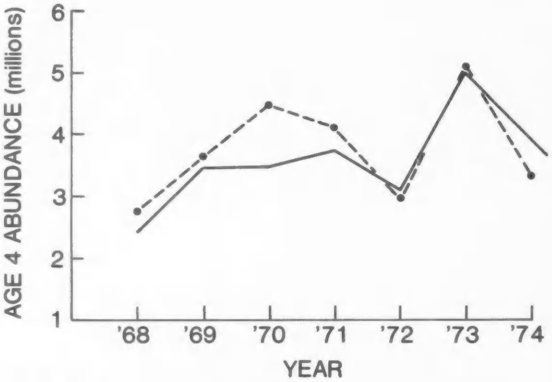

FIG. 3. Time series plot of historical (solid line) and Version 9 (broken line) age 4 abundances, in relation to calendar year when those abundances were estimated.

population's reproduction, growth, and mortality as noted in previous sections.

Unfished equilibrium

We also determined the unfished equilibrium biomass (B_{eq}) for each model version by simulating for 80 yr using randomly chosen years of oceanographic conditions and no fishing mortality. B_{eq} was calculated as the adult (age 4–13) biomass averaged over the last 20 yr of the 80-yr run.

Evaluation criteria

Three criteria were used to evaluate the relative performance of these different models, two related to age 4 abundances and one to the unfished equilibrium biomass of the adult ages (4–13) in the population. We informally weighted the criteria as described below but we did not calculate a formal, combined function for ranking versions of the model. Comparisons of model and observed age 4 abundances were made over the last 7 of the 11 yr of the simulations because only in these last 7 yr were the age 4 abundances generated by the model, rather than arising from the input starting age structure. *Criterion 1*: The heaviest weighting (50%) was put on the correlation between historical and simulated age 4 abundance because this correlation indicates how well the model represents the natural processes which lead to interannual variability in recruits. *Criterion 2*: The next most important criterion (weighted 30%) was the sum of squared deviations (SS) between historical and simulated age 4 abundance *during the last 3 yr* of historical data. Calculation of a correlation coefficient was inappropriate for only three points. The last 3 yr were chosen rather than the 7 in criterion 1 for two reasons. First, if there were major errors in the model components, then the largest errors would appear near the end of the simulated time series when the simulated brood classes, rather than the starting population, would contribute most heavily to simulated age 4 abundances. Second, these last 3 yr in the historical record included the highest and next-to-lowest age 4 abundances and these years were therefore most likely to show differences between model versions which differed in the assumed degree of density-dependent larval mortality. *Criterion 3*: A reasonable B_{eq} was also necessary but our uncertainty concerning its true value led to a relatively low weighting (20%) on this criterion. The B_{eq} sought was 2–5 times the historical fished population biomass of 2000–4000 metric tons (Hayman et al. 1980).

Versions of the model

Versions 1–9 of the model included the various components noted in previous sections, roughly in order of increasing complexity (Table 2). Comparisons among the different versions enabled us to draw conclusions about the likely importance in the field of various biological processes.

Results

As the model increased in complexity, its performance generally improved (Table 4). Version 1, which included the simplest assumptions regarding juvenile mortality, explained only 27% of the interannual variation in age 4 abundance (r = 0.52). Version 7, which included all of the prerecruit components but had density-independent larval mortality, had a correlation of 0.78 and explained 61% of the variation in recruitment (Table 4). However, it gave an unreasonably high B_{eq} (43 000 metric tons) because density-dependent age 1

TABLE 4. Statistics comparing nine versions of the model in terms of their ability to reflect historical data. The second column describes the components included in each version (see Table 2). The first evaluation criterion is the correlation between simulated and observed age 4 abundances over the full 7-yr data set. Next is the sum of squared deviations (SS) between the simulated and observed age 4 abundances in the last 3 yr of the data set. A dash under the equilibrium unfished adult population biomass, B_{eq}, indicates that no equilibrium was generated because of the lack of any density-dependent component.

		Evaluation criteria			
		Correlation full 7 yr		SS last 3 yr	B_{eq} (thousand
Version	Components	r	$P_{r=0}$	(millions)	metric tons)
1	Base case	0.52	0.23	0.81	—
2	Base + juvenile duration	0.63	0.13	0.81	—
3	Base + density-dependent growth	0.52	0.23	0.82	43
4	Base + age-dependent mortality	0.52	0.23	0.80	—
5	Base + density-dependent larval mortality	0.45	0.31	1.55	13
6	Base + juvenile duration + density-dependent growth	0.64	0.12	1.11	43
7	Base + juvenile duration + density-dependent growth + age-dependent mortality	0.78	0.04	0.96	43
8	Base + juvenile duration + density-dependent growth + density-dependent larval mortality	0.61	0.15	0.74	17
9	Base + juvenile duration + density-dependent growth + age-dependent mortality + density-dependent larval mortality	0.78	0.04	0.37	16

growth was the only limit on population biomass. The correlation between observed and model age 4 abundances was similar for Versions 7 and 9. However, Version 9 was closer to historical values in the most important last 3 yr (Fig. 3), giving SS 62% smaller than Version 7. In addition, Version 9 with density-dependent larval mortality resulted in a reasonable B_{eq} of 16 000 metric tons, about 5 times the mid-1960s to 1970s abundance (Hayman et al. 1980).

We therefore chose Version 9 as the final version of the model with which most of our subsequent analysis was done (Peterman and Bradford 1987b). The catches generated by this version were also well correlated with the historical data (Hayman et al. 1980) (numbers of fish caught: $r = 0.87$, $P = 0.011$; catch biomass: $r = 0.95$, $P = 0.001$).

Discussion

The comparisons of several versions of our model with the historical data contributed to our understanding of the important biological processes affecting *Parophrys vetulus* prior to recruitment to the fishery. For example, the incorporation into our model of a variable length juvenile period noticeably increased the proportion of variation in age 4 abundance accounted for (cf. Version 2 with 1 in Table 4). The explanatory power increased because of the high mortality rate on juveniles and the variability between years in timing of spawning, which affects the mean duration of the juvenile life stage in cohorts from different years. Versions 2–5 each added one component to the baseline Version 1, and Version 2 with variable duration of the juvenile period was the only one of these cases which increased the correlation between simulated and observed age 4 abundance (Table 4). Second, the inclusion in Versions 7 and 9 of a decreasing mortality rate with increasing age (prior to age 4) also increased the model's predictive power compared with otherwise identical Versions 6 and 8, respectively (Table 4). This suggests that this type of function may be important in English sole, as has been shown for other species (Cushing 1975; Smith 1985). Third, the similarity in predictive power of Versions 7 and 9 (Table 4) suggests that density-dependent larval mortality was not significant *over the range of abundances encountered in our historical data set*. In the more important last 3 yr of the data set, the smaller forecasting errors of Version 9, which included density-dependent larval mortality, provide weak but noteworthy evidence that such density dependence could further affect larval mortalities at more extreme abundances. This conclusion is consistent with Shepherd and Cushing (1980) and Lockwood (1980) who argued that strong density dependence is likely to occur in fish stocks, especially in the early life stages as postulated here. Finally, density-dependent growth at age 1 does not appear to significantly affect abundance of recruits, through altered reproductive potential, for example. Versions 1 (without density-dependent growth) and 3 (with density-dependent growth) had almost identical correlations between simulated and observed age 4 abundance (Table 4). This held true for Versions 2 and 6 as well. However, we probably underestimated the importance of density-dependent growth because, due to lack of data, we had to assume that fish at the beginning of age 1 were the same size in all years. This was probably not true in the field; small fish probably suffered more mortality than large fish. Inclusion of this effect of size on mortality rate might further increase our ability to explain the interannual variation in abundance of age 4 fish.

There is some circularity in our comparisons between historical data and various versions of the model; the same data set was used to estimate some model parameters and to compare with simulated output. However, this is not a serious problem because most of the processes which cause interannual variation in recruitment, such as growth, spawning timing, and egg hatching, were estimated *independently* of the historical age 4 abundances used for model testing. The one exception was the selection of the two density-dependent larval mortality parameters, which was done by comparing model and historical abundances. We must also emphasize that this was not an exercise in fitting a multiple regression or polynomial model. Because parameters of most components were estimated independently of the age 4 abundance data, adding more complexity to the model could have just as easily decreased the proportion of variation accounted for in age 4 abundance, as increased it.

Our final version of the English sole model accounts for 61% of the interannual variation in age 4 abundance whereas Kruse's (1983) model accounted for only 53%, over a slightly longer time series. While both models included similar components for egg production, spawning timing, and egg hatch success, Kruse's model attempted to explain interannual variation in recruitment by focusing mainly on the influence of physical oceanographic factors such as advection on the survival rate of larvae. As well, Kruse did not model the dynamics of the post-larval period, but rather, juvenile and prerecruit survival were assumed to be constant. His resulting age 4 recruits also had no effect on the subsequent spawning population because each year's adult abundances were put in as data. In contrast, we simulated the population dynamics of the full life cycle, incorporating the effects of survival through the larval and juvenile stages on resulting adult abundances and their subsequent egg production. Major errors in our assumed early life history mechanisms would therefore have led to errors in numbers of adults, their resulting eggs, and subsequent production of age 4 fish. The high correlation between simulated and historical age 4 abundance in our Version 9 ($r = 0.78$) therefore lends some credibility to the biological mechanisms included in our model. The post-hatch components included in our model and Kruse's (1983) are not mutually exclusive; the addition of Kruse's advection process may have improved our model's performance, but we chose to focus here mainly on biological feedback mechanisms.

The empirical components approach to model building that we have used allows us to more fully investigate the biological mechanisms causing interannual variation in recruitment than is possible with correlation analysis. The explicit incorporation of a temperature-dependent egg hatch success rate in the model enabled us to split Hayman and Tyler's (1980) hypothesis regarding the effects of spawning timing on recruitment into pre-and post-hatch components. While our model and that of Kruse (1983) both show that spawning timing does affect recruitment through egg hatch success, our model also indicates an additional effect due to the differential survival of early- and late-spawned juveniles.

We included a density-dependent larval mortality term in the model based on the assumption that at some point there must be population regulation. It is not surprising that researchers have had difficulty in detecting density-dependent mortality in the field; population homeostasis is achieved with very small increases in mortality. For example, if the sole stock doubled in abundance over current levels, the instantaneous total mortality over the egg-to-age 4 period in our final model would only increase from 12.39 to 12.71, a difference that would be difficult to measure in the field.

As mentioned earlier, the purpose intended for our model was for a Monte Carlo simulation study of population monitoring and experimental management schemes. For this use we required a population model which emulated processes which lead to the observed variability in recruitment. While we do not have any strong evidence that the hypotheses included in our model are more correct than those used by Kruse (1983) or other alternatives, our final model is acceptable for our purpose because it adequately reflects the net effect of variability in ocean conditions on interannual variability in recruitment. However, we are not justified in using the model to make management recommendations about harvest rates or catch levels. Although the model is internally consistent because the model components and the tests of them used data taken over the same time period, there is recent evidence that long-term changes in growth schedules are occurring (E. Pikitch, pers. comm.) A management model would require the incorporation of these processes.

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Appendix: Juvenile and Age 0–3 Mortality Rates (M_J, M_0–M_3)

The juvenile and age 0–3 mortality rates were estimated by first calculating the total mortality during the period from the beginning of larvae to the end of age 3 (M_{L-3}) from the 6 yr of complete historical data (1965–70). Then for the alternative versions of the juvenile component, we separately estimated the portion of that total mortality which occurred during two periods: juvenile (postmetamorphosis) and age 0–3.

M_{L-3}

To obtain the total mortality, female larval abundances for these years were calculated as the product of the total egg production, from cohort analysis of female stock sizes (Kruse 1983) and the egg production algorithms discussed above, an average egg survival rate of 0.66, and a 50:50 sex ratio. The egg survival rate of 0.66 is the geometric mean of the egg survivals calculated over 23 yr (1954–76) from historical monthly temperatures, monthly spawning distributions as estimated by Kruse and Tyler (1983), and Alderdice and Forrester's (1968) temperature-dependent egg survival relation. The abundance of age 4 females 4+ yr after metamorphosis was estimated by cohort analysis (Kruse 1983). The total mortality from the beginning of the larval period to the end of age 3, M_{L-3}, was then calculated for each of the six complete year-classes, and these values were averaged to give $M_{L-3} = 12.39$ (Table A.1).

Mortality for the juvenile-to-end-of-age 3 period was calculated by subtracting 2 mo of larval mortality (monthly $M_L = 1.209$) from M_{L-3} to give $M_{J-3} = 9.97$.

TABLE A.1. Data used in the calculation of the total mortality from start of the larval period to start of age 4 (sources noted in the Appendix). Year refers to year class; for example, 1965 corresponds to the 1964–65 spawning season. Number of female larvae was calculated as the product of the number of eggs, an average egg survival rate of 0.66, and a sex ratio of 0.50.

Year	10^{13} eggs	10^{12} female larvae	10^3 female age 4	M_{L-3}
1965	0.208	0.686	3449	12.20
1966	0.238	0.785	3466	12.33
1967	0.327	1.079	3728	12.58
1968	0.296	0.977	3080	12.66
1969	0.292	0.964	5001	12.17
1970	0.282	0.931	3891	12.39
			Average M_{L-3} =	12.39

M_J and M_{0-3}

A. Fixed-length juvenile period

In the simplest versions tested (1, 3–5), the duration of the juvenile period was assumed to be 6 mo, and the monthly mortality rate was assumed to be constant over the entire juvenile-to-age 3 period. The total juvenile-to-age 3 mortality of 9.97 derived above was therefore divided evenly over the 4.5-yr period, resulting in a 67% mortality rate during the juvenile period and a 89% mortality rate during each year for ages 0–3.

B. Variable-length juvenile period

In all other versions of the model (2, 6–9), we calculated a monthly juvenile mortality rate and applied it each month that a given monthly cohort was in the juvenile stage. This variable length of the juvenile period was accounted for in the following way in our parameter estimation procedure. The survival of a monthly cohort of fish from the larval stage to the end of age 3 can be represented as $S_{L-3} = S_L S_{JT} S_{0-3}$, where the subscripts refer to the fractional survival rate in the larval through age 3 (L–3), larval (L), total juvenile (JT), and age 0 through 3 (0–3) periods.

1. Age-independent mortality rates — In one set of versions (2, 6, and 8 in Table 2), monthly juvenile and age 0–3 mortality rates were equal and independent of age, so that $S_{0-3} = S_J^{48}$, where S_J is the monthly juvenile survival rate and $S_{JT} = S_J^{i}$, where i is the number of months the fish spend in the juvenile phase. The survival rate of an annual cohort is then

$$(1)\quad S_{L-3} = S_L\left(\sum_{i=1}^{12} S_J^{i+48}\, p_i\right)$$

where p_i is the proportion of a year's production of juveniles that has the survival rate of S_J^{i}. The vector of p_i values depends on the monthly spawning distribution for each year. This equation was rearranged as

$$\sum_{i=1}^{12} S_J^{i+48}\, p_i - S_{L-3}/S_L = 0$$

and the monthly fractional survival rate (S_J) was estimated for each year using a polynomial solving package (IMSL 1982). For this estimation procedure, S_L was 1.209 as noted previously and S_{L-3} was derived from the mortality rate estimated in Table

A.1. The 23 yr of monthly spawning timings (Kruse and Tyler 1983) enabled us to derive 23 estimates of S_J. An instantaneous monthly juvenile mortality rate of 0.188 was estimated from the geometric mean of these 23 survival rates.

2. Age-dependent mortality rate — In versions 7 and 9 of the model described in Table 2, we assumed that mortality rate decreased with age from juveniles through age 3 and that the juvenile period had variable duration. The age 0–3 annual mortality rates were estimated first, and then the polynomial fitting procedure was used to estimate the monthly juvenile mortality rate, as described below.

To calculate the age 0–3 mortality rates, we fit an exponential decay model of the form $M = ce^{-dT}$ to the mortality over the period from metamorphosis to age 4, where M is the annual mortality rate for fish of age T. The parameters were chosen using the constraints that (1) the total area under the exponential curve should be equal to 9.97, the average total mortality between metamorphosis and age 4 as estimated above, and (2) $M_4 = 0.294$, the adult mortality rate estimated by Hayman et al. (1980). The resulting mortality rates for ages 0–3 are shown in Table 1.

Equation (1) above was then modified to:

$$S_{L-3} = S_L\left(\sum_{i=1}^{12} S_J^i\, p_i\right) S_{0-3}$$

where S_{0-3} is the age 0–3 survival rate derived from the mortality rates calculated above (Table 1). The procedures described earlier were used to derive the estimate of monthly M_J given in Table 1, 0.727.

Statistical Power of Trends in Fish Abundance

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Peterman, R. M., and M. J. Bradford. 1987. Statistical power of trends in fish abundance. Can. J. Fish. Aquat. Sci. 44: 1879–1889.

Estimation errors inherent in stock assessment methods may make it difficult to estimate time trends in fish abundances correctly. Our objective was to quantify the probability that trends in abundance of recruits will be successfully identified. For this analysis, we used an empirically based simulation model of English sole (*Parophrys vetulus*) off the west coast of North America. The unique wealth of data and past analyses of this population permitted us to include deterministic and stochastic components of growth, mortality, and reproduction in a realistic manner. Errors were also included in two simulated stock assessment methods: a trawl survey and cohort analysis. Under various conditions, we calculated the probability (analogous to statistical power) that these methods will meet three management objectives concerning time trends in recruitment. Monte Carlo simulations showed that although power depends on the objective, under most realistic conditions the probability of correctly detecting recruitment time trends may be unacceptably low. These results suggest new management guidelines for fisheries.

À cause des erreurs d'estimation inhérentes aux méthodes d'évaluation des stocks, il peut être difficile d'estimer correctement les tendances temporelles en ce qui a trait à l'abondance des poissons. Notre objectif était d'évaluer la probabilité de bien déterminer les tendances relatives à l'abondance des recrues. À cette fin, nous avons utilisé un modèle empirique de simulation construit pour la population de la sole anglaise (*Parophrys vetulus*) du large de la côte ouest de l'Amérique du Nord. L'abondance des données et les analyses déjà effectuées sur cette population nous a permis d'inclure des composantes déterministes et stochastiques réalistes pour la croissance, la mortalité et la reproduction. Nous avons également inclus des erreurs dans des évaluations simulées des stocks par deux méthodes : un relevé au chalut et une analyse de cohortes. Nous avons calculé pour diverses conditions la probabilité (analogue à la puissance statistique) que ces méthodes répondent à trois objectifs de la gestion concernant les tendances temporelles du recrutement. Des simulations utilisant des méthodes de Monte-Carlo ont indiqué que même si l'efficacité (puissance) dépend de l'objectif, dans la plupart des conditions réalistes, la probabilité d'établir correctement les tendances temporelles du recrutement risque d'être trop faible. D'après ces résultats, de nouvelles lignes directrices pour la gestion des pêches seraient nécessaires.

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Estimation of time trends in population abundance is an important component of stock assessment in fisheries management. In some cases, particularly for marine mammals, a common management goal is to determine simply whether abundance is increasing, decreasing, or remaining stable (DeMaster et al. 1982; International Whaling Commission 1983; Marine Mammal Protection Act 1984). In other cases, scientists have proposed deliberate, experimental exploration of how populations with unknown underlying dynamics will respond to different fishing regimes (Walters and Hilborn 1976; Allen and Kirkwood 1976; Holt 1977; Tyler et al. 1982). In these latter situations, often referred to as cases of adaptive management, optimal fishing regimes can, in theory, be determined by monitoring trends in population responses. In both of the above types of situations, which we call, respectively, monitoring or experimental fishing programs, our ability to detect changes in population abundances will depend on the demographic parameters of the stock, natural variability in growth, survival, and reproduction, and the accuracy and precision of the stock assessment methods.

Our purpose was to apply standard concepts of statistical power analysis (Cohen 1977), which are used in experimental design, to these types of monitoring programs or large-scale field manipulations of fish populations. In laboratory experiments, scientists know that they cannot draw a valid conclusion if they fail to reject the null hypothesis, unless the experiment had high statistical power (e.g. large sample or large treatment effect). Similarly, fisheries managers should be aware of the power of their monitoring programs or manipulations before drawing conclusions from resulting data.

In this paper we show quantitative examples of power in three situations which exist in fisheries management. Using an empirically based simulation model, we explore how various factors affect the creation of time trends in abundance of recruits and the ability of stock assessments to discern those trends. These factors were fishing mortality rate, the duration of the monitoring program or experiment, and the magnitude of errors in methods of stock assessment. We determined the minimum number of years that annual stock assessments would be needed at a given fishing mortality rate in order to provide an acceptably high probability (≥0.8) that estimated trends correctly reflect true trends.

The general trends of the effects of sample size and fishing intensity found here were expected from basic statistical theory.

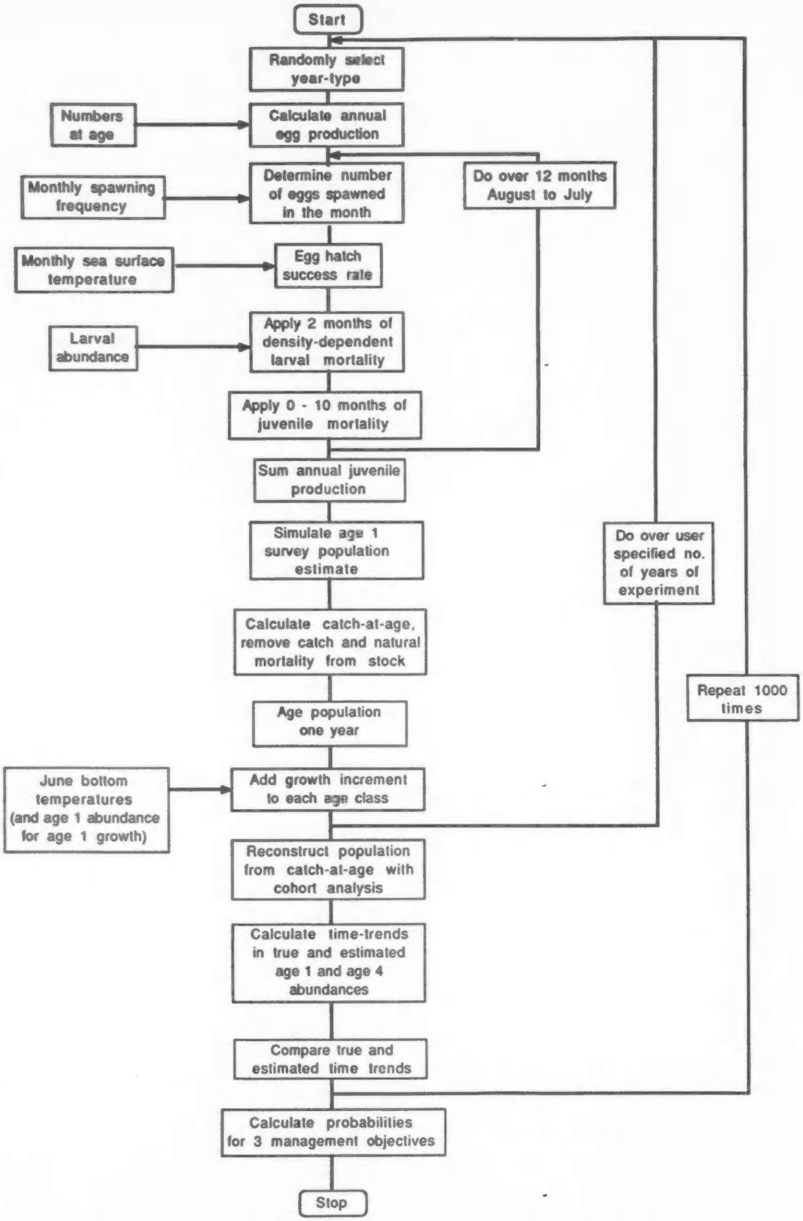
Start
Randomly select year-type
Numbers at age
Calculate annual egg production
Monthly spawning frequency
Determine number of eggs spawned in the month
Do over 12 months August to July
Monthly sea surface temperature
Egg hatch success rate
Larval abundance
Apply 2 months of density-dependent larval mortality
Apply 0 - 10 months of juvenile mortality
Sum annual juvenile production
Simulate age 1 survey population estimate
Do over user specified no. of years of experiment
Calculate catch-at-age, remove catch and natural mortality from stock
Repeat 1000 times
Age population one year
June bottom temperatures (and age 1 abundance for age 1 growth)
Add growth increment to each age class
Reconstruct population from catch-at-age with cohort analysis
Calculate time-trends in true and estimated age 1 and age 4 abundances
Compare true and estimated time trends
Calculate probabilities for 3 management objectives
Stop

FIG. 1. Flow chart for the Monte Carlo simulation of English sole population dynamics.

However, the value of our analysis was that it quantified the specific probabilities that would result from a complex population model. Our model, which included age structure, negative feedbacks, and strong linkages with environmental variation, allowed for more accurate estimation of power than the simple models used by Allen and Kirkwood (1976) and de la Mare (1984).

For our analysis we used a simulation model of English sole (*Parophrys vetulus*) off the west coast of North America because of the unique wealth of data which describes the deterministic and stochastic components of this species' population dynamics (see Peterman et al. 1987). These data permitted us to include natural variability realistically in our model. The population represented in the model occupies the portion of the Washington and Oregon coast designated area 3A by the Pacific Marine Fisheries Commission (45°46′N to 47°20′N).

We emphasize that we used the English sole case merely as a prototype to address in a realistic way the more general problem

TABLE 1. Baseline parameter values used in the stochastic English sole model.

Symbol	Value	Definition
a	1.05/mo	Intercept of density-dependent instantaneous monthly larval mortality function
b	6.4×10^{-13}	Slope of density-dependent larval mortality function
M_J	0.727/mo	Instantaneous monthly juvenile mortality rate
M_0	3.551/yr	Instantaneous annual natural mortality (age 0)
M_1	1.904/yr	Instantaneous annual natural mortality (age 1)
M_2	1.021/yr	Instantaneous annual natural mortality (age 2)
M_3	0.548/yr	Instantaneous annual natural mortality (age 3)
M_A	0.294/yr	Instantaneous annual adult natural mortality rate (ages 4–13)
σ_M	0.05	Standard deviation in age 0–13 natural mortality
σ_X	0.4	Standard deviation in age 1 trawl survey estimates
CV_F	0.2	Coefficient of variation in fishing mortality
R_{0-3}	0.0	Ages 0–3 partial recruitment coefficients
R_4	0.5	Age 4 partial recruitment coefficient
R_{5-13}	1.0	Ages 5–13 partial recruitment coefficients

of estimating fish population trends in variable habitats with imprecise methods. We are *not* attempting to make management prescriptions for the specific flatfish population used in our example. Instead, we emphasize results that are generally applicable to other species.

Components of the Model

In the next few sections, we briefly describe the main components of the Monte Carlo model, showing how they were modified from our original model (version 9 of Peterman et al. 1987). Figure 1 is a flowchart of the modified model, and Table 1 includes the parameter values used for the baseline simulations. Here, as in Peterman et al. (1987), a year refers to the 12-mo period beginning August 1.

Oceanographic Components

Several biological processes are influenced in the model by sea surface or sea bottom temperatures. We used the 24-yr data series on these temperatures from Kruse (1983) and Kruse and Huyer (1983). At the beginning of each simulated model year, one of the 24 historical years was randomly chosen from a uniform distribution and the monthly surface and bottom temperatures from that historical year (henceforth called the year-type) were used for that model year. By randomly choosing years from the historical data, we assumed that oceanographic conditions in each year were independent of events that occurred in previous years. Before making this assumption, we did autocorrelations to test whether the temperature in any month was related to temperature in the same month of previous years, where the lag was varied from 1 to 12 yr. Two of the 144 (12 mo by 12 yr lags) autocorrelation coefficients were significant at $\alpha = 0.05$ for both sea surface and bottom temperatures. However, we would expect at least this many significant correlations by chance alone, even if there were no real correlation. Therefore, we made the assumption of independence of temperatures across years. Monthly surface and bottom temperatures were taken from the same randomly chosen year-type, however, to preserve any covariance that may exist within a year.

Biological Components

The model and the data used to derive its parameter estimates are described in detail in Peterman et al. (1987), but a brief overview of its empirically derived components is required here. Timing of spawning within each simulated year was influenced by ocean bottom temperatures, and fecundity as well as proportion of females maturing were functions of fish length. The fraction of the resulting eggs that hatched was a dome-shaped function of sea surface temperature, and survival of the resulting sole larvae was density dependent. Mortality rates of juveniles and fish up to age 4 decreased with increasing age, and natural and fishing mortality of adults were calculated as described below. Age-dependent growth rates were functions of water temperature (plus abundance for growth of age 1 fish only).

The submodels for spawning, egg production, larval and juvenile survival, and growth were unchanged from the final model of Peterman et al. (1987), with the exception that these components were driven with the randomly chosen oceanographic data described above, rather than the historical sequence used in Peterman et al. (1987). Interannual variation in abundance of year classes thus resulted from complex interactions among environmental variables, stock abundance, and density-dependent processes.

Mortality (natural and fishing)

To simulate some of the natural variability which occurs in field populations, we added random noise to the natural mortality rates given in Peterman et al. (1987) for ages 0 to 13. The actual instantaneous annual mortality rate applied to each age was $M_i^* = M_i + V$, where V is a normal random deviate with mean 0 and $\sigma = 0.05$ and M_i is the deterministic age-specific natural mortality rate shown in Table 1. This meant that 95% of the simulated M_i values for adults were in the range of 0.2–0.4.

English sole catches by the trawl fleet consist primarily of age 4 and older females (Hayman et al. 1980). Fishing mortality estimates from cohort analysis of 1963–77 catch data indicate that the fishing mortality of age 4 fish was approximately half that of the age 5 and older classes (Hayman et al. 1980). Therefore, we assumed in the model that the partial recruitment coefficient was zero for ages 3 and below, 0.5 for the age 4 fish, and that older age groups were fully recruited to the fishing gear.

Instantaneous annual fishing mortality rate was a user-supplied input in the model. A random noise term was added to the input fishing mortality rates across all ages to simulate fluctuations in realized mortality that might be due, for example, to variation in catchability, q. The realized fishing mortality was normally distributed, with a mean of the input F and a coefficient of variation of 0.2. The use of a constant coefficient of variation rather than a constant standard deviation is based on the argument that nominal fishing effort, f, is closely regulated in an experimental management program such as the one simulated here. Since fishing mortality $F = qf$ (Ricker 1975), fluctuations in F will depend largely on fluctuations in catchability across all ages. Therefore, absolute variation in F will increase linearly with nominal effort. In fact, variability in effort may also increase with increasing f, in which case the variation in F may be underestimated at high f by the constant coefficient of variation in the noise term.

The realized age-specific natural and fishing mortality rates were used in the Baranov catch equation (Ricker 1975) to calculate the catch and survivors for each age in each year.

Components of Simulated Stock Assessment

In our simulated field situations, nominal fishing effort was held constant, and managers estimated age 1 abundance by trawl survey and age 4 abundance by cohort analysis. The former represents the commonly used but highly uncertain real-time survey of prerecruit abundance of a year class (Doubleday and Rivard 1981), and the latter is the abundance of the first recruited age class as estimated by a relatively precise but delayed method (Pope 1972). Time trends in year class strength can provide a useful adjunct to other data used by fisheries managers because such trends may reflect long-term changes in reproductive output of a stock and hence future surplus production.

In this paper, we use "true" abundances to mean the abundances simulated by the population model, which include the effects of variability in natural and fishing mortality, timing of spawning, and body growth. These "true" abundances are distinct from the abundances "estimated" by the simulated stock assessments described below. The "estimated" abundances reflect the imprecision of the simulated estimation methods (trawl survey and cohort analysis), which introduce "noise" on top of the natural variability which already exists in the "true" abundances. Thus, time trend regressions fit to "true" abundances will differ from regressions fit to "estimated" abundances, and it is this difference which is the focus of this paper.

We simulated trawl survey estimates of abundance of age 1 fish by applying a random sampling noise term to the true total age 1 abundance, N_T, simulated by the model. Empirical and theoretical evidence indicates that trawl survey catches are log-normally distributed within a year (Doubleday 1981, Leaman 1981). In the model, the annual trawl survey abundance estimates, N_S, were obtained from $N_S = N_T e^X$, where X is normally distributed with mean zero and a variance $\sigma_X{}^2$. The baseline value of $\sigma_X{}^2$ was set by examining estimates of the variability in bottom trawl survey population estimates given in Doubleday and Rivard (1981). The arithmetically calculated coefficients of variation (CV) given in Doubleday and Rivard (1981) were approximated by using the CV of the log-normal distribution: $\mathrm{CV} = (e^{\sigma^2} - 1)^{0.5}$ (Aitchison and Brown 1963). We used a baseline σ_X of 0.4, which corresponds to a CV of 0.42; this is in the middle of values found in Doubleday and Rivard (1981). Logarithms were taken of N_T and N_S before comparisons were made of time trends in true and estimated abundances; therefore, $\log_e N_S$ is an unbiased estimator of $\log_e N_T$.

The abundance of age 4 fish was estimated from the entire simulated catch-at-age matrix using Pope's (1972) cohort analysis. The inputs for cohort analysis (M for ages ≥ 4, F of the oldest age, and the partial recruitment coefficients) are typically estimated from historical data or through independent methods (Rivard 1983). The deterministic natural and fishing mortality rates were used by the cohort analysis in the model, since these values are the average of the stochastic rates applied in the simulated population. Cohort analysis was performed once at the end of each simulated experiment to estimate the abundance of age 4 fish in each year of the experiment.

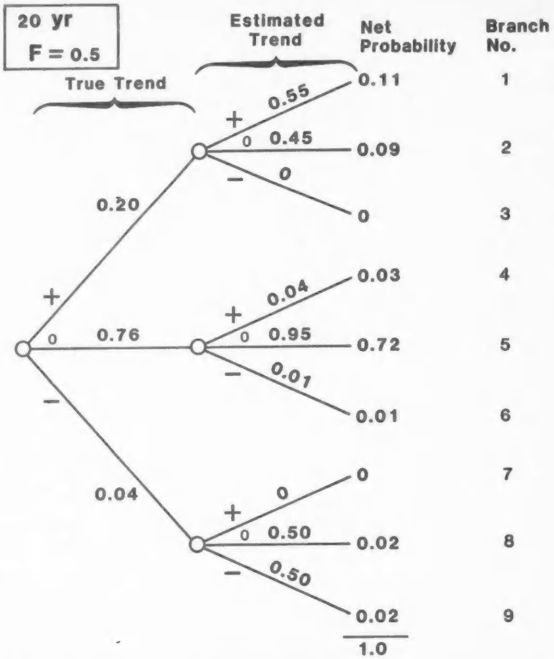

FIG. 2. Example tree of possible outcomes from 1000 simulations run for 20 yr each at $F = 0.5$. The category of a time trend in age 1 abundance is indicated by + (significant increase), − (significant decrease), or 0 (not significantly different from 0). Fractions on each branch of the tree are the proportions of the 1000 cases which were in the category indicated for the true age 1 population trend (left column of probabilities) or estimated age 1 trend (middle column). Net probabilities of being on one of the nine numbered branches are shown in the third column.

Design of Simulated Experiments

We determined by Monte Carlo simulation the conditions which would give high power, i.e. high probability of achieving the goals of monitoring or experimental management, outlined below. This probability was calculated separately for the trawl survey and for cohort analysis. To simplify discussions, we chose a probability of 0.8 as being an acceptably high value; use of another power value would not change our general conclusions. The conditions which would give high power are described by four factors controlled by fisheries managers. First is the level of α used in tests for significant time trends; we used the standard $\alpha = 0.05$. Second, fishing mortality affects power because it influences the rate of change in true abundance; faster rates of increase or decrease in abundance will increase the probability that a trend will be detected in spite of the errors inherent in a stock assessment method. Third, duration of a monitoring program also affects the probability of correctly detecting trends because increased sample size permits better estimation of trends, thus increasing power (Sokal and Rohlf 1969). Fourth, the precision and accuracy of stock assessment methods clearly influence their power to the extent that true trends tend to be masked by estimation errors. We used our English sole model to quantify how power was affected by the last three factors: fishing mortality, duration of the program, and errors in stock assessment methods.

We compared simulated time trends in abundance of recruits ("true" trends) with trends estimated by our simulated stock assessments ("estimated" trends) in order to evaluate a fishery manager's ability to correctly detect population trends. Estimated trends were those derived from our simulated age 1 trawl survey and from the cohort analysis of age 4 abundances, which was based on simulated catches. We fitted linear time trends by regression to "true" and "estimated" abundances of each of age 1 and age 4 fish. We took natural logs of age 1 and age 4 abundances before fitting time trends to normalize residuals because frequency distributions of recruitment indices are log-normally distributed for marine fish species (Hennemuth et al. 1980; Garrod 1983). True and estimated regression slopes were separately tested for statistical significance. The categories of significance (significantly increasing, significantly decreasing, or not significantly different from zero) for true and estimated trends were compared, as described in the next section. What we call a "correct detection or identification" of a population trend was where the category of significance of the estimated trend was the same as the category for the true trend (e.g. both were significantly decreasing).

The model was run at each combination of nine mean instantaneous annual fishing mortality rates (ranging from $F =$ 0.1 to 1.0 on fully recruited ages) and 11 durations of the program (ranging from 4 to 20 yr). Each of the 99 combinations of F and duration are referred to below as "fishing plans" because fishery managers can affect those two characteristics. For each combination of F and length of the program, 1000 replicates were run; each replicate had a different series of random numbers. The significance levels of the true and estimated slopes for the time trends in abundance were calculated for each replicate. The baseline parameter values in Table 1 were used for each year and each replicate, unless stated otherwise. These steps in our Monte Carlo analysis are summarized in Fig. 1.

Management Objectives

The method of determining preferred fishing plans depended on the management objective. We explored three different objectives to increase the generality of our results, even though these objectives are not currently employed in management of our English sole population (again, the sole model is merely an example).

Objective 1

The first management objective was to impose a reduced fishing mortality rate for a sufficiently long period to create a high probability of obtaining a significant increase in both true and estimated recruitment. This objective might be used with a population which was known to be well below the abundance which would generate maximal or optimal sustained yield. An increase in true recruitment would be desirable because it would permit recovery of the population to a more productive level where surplus production might be larger. The program would also need sufficient duration to generate a high probability that the *estimated* time trend in recruitment correctly reflected an increasing *true* trend. Thus, we used the probability of drawing a correct conclusion about the significance of the true trend in combination with the goal of generating an increasing true trend in abundance.

To quantify how well each fishing plan met this objective, we calculated its "success rate." A high rate of success for a given experimental regime was reflected by a high probability of concurrently generating a statistically significant increasing time trend in both true and estimated recruitment abundances. This success rate is called the power of the experiment for management objective 1. To show how this performance indicator was calculated by our simulations, an example "branching tree" of possible outcomes is given in Fig. 2. In this figure, a fishing plan with fishing mortality on the oldest ages of $F = 0.5$ and a 20-yr duration had the probabilities P shown at the left of the figure of generating a significant positive time trend in *true* abundance of age 1 fish ($P = 0.2$), no significant trend ($P = 0.76$), or a significant decreasing trend ($P = 0.04$). These probabilities were simply the proportion of 1000 different stochastic simulations of this fishing plan which generated those particular time-trend results. The example fishing plan in Fig. 2 also had the probabilities shown of having +, 0, or − *estimated* time trends in age 1 abundance. The "success rate" for this experimental fishing plan was the joint probability of obtaining a significant positive trend in both the true and estimated abundances ($(0.2 \times 0.55)/1.0 = 0.11$). This success rate was calculated for a series of different F values and durations of experiments. Similar calculations were made for age 4 abundances.

Objective 2

This objective differs from the first in that we do not necessarily want to *take an action* that will increase recruitment. Instead, we simply want to estimate abundance for enough years so that if population estimates indicate a significantly increasing trend, then there is a high probability that a significant increase in true recruitment also exists. In other words, in such situations we want to be able to state with a high degree of confidence that true abundance of recruits has increased, i.e. we want high power. We define power here for the second management objective as the probability that an increasing estimated trend has correctly identified the true trend. An objective such as our second one exists in marine mammal monitoring programs, where a legislated goal is simply to assess time trends (Marine Mammal Protection Act 1984). Pinnipeds on the coast of California are a good example because they are perceived by the sport and commercial fishing industries as competitors for fish (Jay Barlow and Alec MacCall, National Marine Fisheries Service, La Jolla, CA, pers. comm.). There are preliminary indications that pinniped abundance is increasing, but before any action is taken against the population, stock assessments should be carried out long enough to have a high power for correctly detecting an increasing trend. A short program with low power may increase chances that managers may reach an incorrect conclusion.

Applying this objective to our example model, a fishery manager would want to know, "Given that I am at the end of my monitoring program, and that the data show a significant increase in estimated age 1 (or age 4) abundance over time, what is the probability that the true time trend was also significant and positive?" In the example of Fig. 2, this probability was calculated from Bayes' theorem (Raiffa 1968) as $0.11/(0.11 + 0.03 + 0.0) = 0.79$. This probability differs from the one shown above for the first management objective (0.11) because in the latter case, we calculated the probability of being on a particular branch (the top one), out of *all* the other possibilities. But in the current case, there were three ways in which a significant positive *estimated* trend could have arisen (branches 1, 4, or 7 in Fig. 2), so the divisor was the sum of probabilities of being on those three branches, and the numerator was the probability of being on the correct branch. Again, calculations were done separately for age 1 and age 4 abundances, and for a range of durations of programs and fishing mortality rates.

Objective 3

This objective is the same as the second, except that the aim is to have a high probability of drawing correct conclusions about decreasing, rather than increasing trends. This objective applies in situations in which an intensively fished population is below its optimum biomass, but managers are under pressure from the fishing industry to continue current fishing regulations. Managers of such stocks usually require convincing documentation of declining recruitment before taking strong action to restrict the fishery (Saetersdal 1980). These managers therefore should know the probability that a significant decreasing trend *estimated* from stock assessments indicates a significant decreasing trend in *true* abundance.

As in objective 2, we used Bayes' theorem to calculate this probability, this time focusing on the three ways in which significant decreasing estimated trends could arise (branches 3, 6, or 9 of Fig. 2). For this example with $F = 0.5$ and a duration of 20 yr, the probability that a significant decreasing trend in the estimated population came from a significantly decreasing actual population was 0.67, which was the net probability of being on the correct branch (0.02), divided by the sum of probabilities of being on branches 3, 6, and 9 (0.03). Of course, more significant digits were used in the simulation.

Initial Abundance

Because of the density-dependent processes in the model, the responses of the population to various fishing regimes depended on the abundance at the start of a simulated experiment. However, the three management objectives above refer to cases in which initial abundance is relatively low. Therefore, we report here only results for simulations which used low initial abundance, which we arbitrarily defined as the average abundance by age during the last 30 yr of a 60-yr simulation with $F = 0.5$. The resulting female abundance was 1090 thousand metric tonnes, or about one third of the average 1964–75 biomass of our example English sole population (Hayman et al. 1980).

Results

Objective 1

The level of fishing mortality, F, had the largest effect on the success of a fishing plan under management objective 1: that objective was to increase the number of recruits and successfully detect the increase by stock assessments. F had a large effect because it influenced the rate of change of the population and consequently the slope of the true time trend in abundance of recruits. Figure 3 illustrates results from a 10-yr experiment. At a low F of 0.1, the abundance of age 4 recruits generally climbed rapidly over time, generating significant increasing true trends in 72% of the cases. In most of these cases, abundance estimates derived from cohort analysis also showed significant increasing trends. The resulting joint probability of success was 63%, meaning that the probability was 0.63 of both generating and correctly detecting a significant increasing trend in age 4 abundance. This success rate (Fig. 3, solid line) decreased rapidly as F increased, largely because of the smaller probability of obtaining a significant increase in true abundance (Fig. 3, broken line).

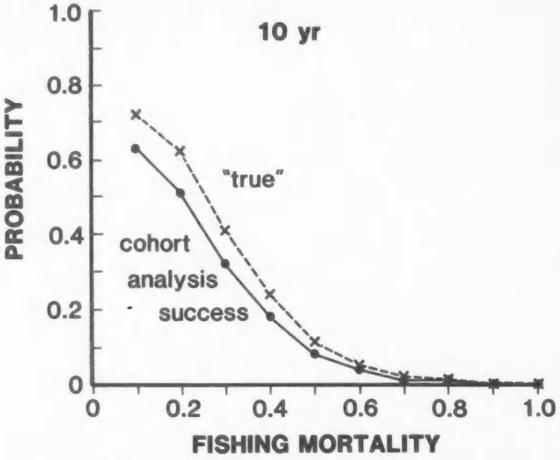

FIG. 3. Probabilities for a simulated 10-yr experiment at various instantaneous annual fishing mortality rates, F. Here and in subsequent figures, F is the rate on the fully recruited ages (5–13); other ages have the partial recruitments shown in Table 1. The broken line is the probability that age 4 recruitment of the "true" population increased significantly. The solid line is the probability of a simultaneous occurrence of a significant "true" trend and a significant increasing trend in estimated age 4 abundance, as estimated by cohort analysis. The solid line therefore represents the probability of meeting management objective 1 by using cohort analysis.

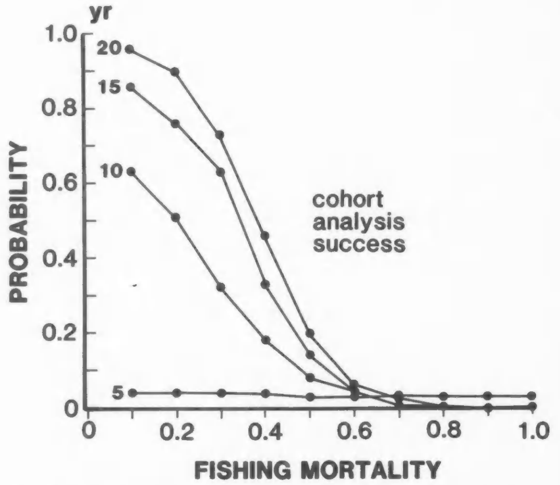

FIG. 4. Probability that cohort analysis will meet management objective 1, for durations of the experiment ranging from 5 to 20 yr.

The duration of a program also affected its probability of success for meeting objective 1. A family of probability curves for different lengths of experiments shows that longer experiments at a given fishing intensity increased their success rate (Fig. 4). This was due to the increased power of test associated

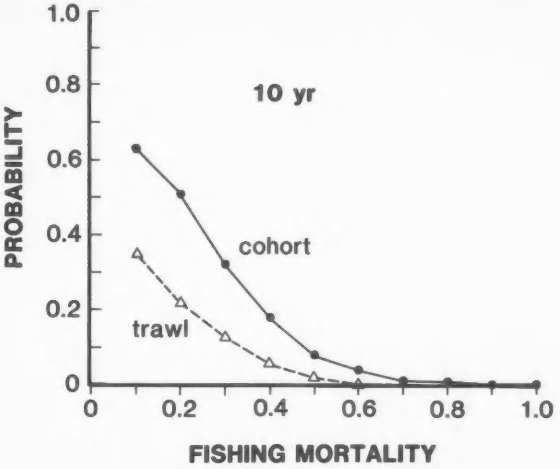

FIG. 5. Probability that cohort analysis (solid line) or trawl survey (broken line) will meet management objective 1 in a 10-yr experiment.

with more annual data points for fitting both true and estimated time trend regressions. If a fisheries manager wants a probability of success of at least 0.8, then none of the experiments lasting less than about 14 yr would be acceptable, if this measure of success for cohort analysis were the sole indicator of the merit of the experiment.

The success rate for trawl surveys of age 1 abundance was not as high because trawls, with their relatively low precision, were not as reliable in assessing the fish population's response to a given fishing regime as cohort analysis (Fig. 5). True age 1 abundances had almost identical probabilities of increasing significantly during an experiment as did true age 4 abundances, so the difference between the two curves in Fig. 5 is a result of the difference in precision of estimates from the two stock assessments.

To facilitate further presentation, we show results as response surfaces. The probabilities for cohort analysis from Fig. 4, along with other durations of experiments, are plotted in Fig. 6B; the analogous isopleths for success rate using age 1 abundance estimates are given in Fig. 6A. No probability values are given for cohort analysis below $F = 0.1$ because zero catches would preclude use of cohort analysis. Trawl surveys are not limited by lack of commercial catch samples.

Starting with a depleted population, only experiments with low F and long duration will have a high probability ($P \geq 0.8$) of both generating and correctly detecting significant increases in recruitment when age 4 abundances are estimated by cohort analysis (Fig. 6B). However, no combination of F and duration of experiment is acceptable if age 1 abundances alone are monitored by trawl surveys (Fig. 6A, no $P \geq 0.8$ on the response surface). For both ages, the probability of generating an increasing true trend declined rapidly with increasing F. Note that because cohort analysis correctly detected most (85–90%) of the true increasing trends (see Fig. 3), the size of the region with $P \geq 0.8$ is almost as large as is possible for this management objective.

Objective 2

In contrast with objective 1, our goal here was to find experiments which gave high probabilities that an observed significant increase in recruitment will have come from a population with significantly increasing true recruitment. Isopleths of Bayesian probabilities for this increasing trend of age 4 abundance showed that the estimated trend was correct more than 80% of the time for most combinations of F and length of experiment (Fig. 7B). However, at least 6 yr of data were needed to achieve that power. If only trawl survey estimates

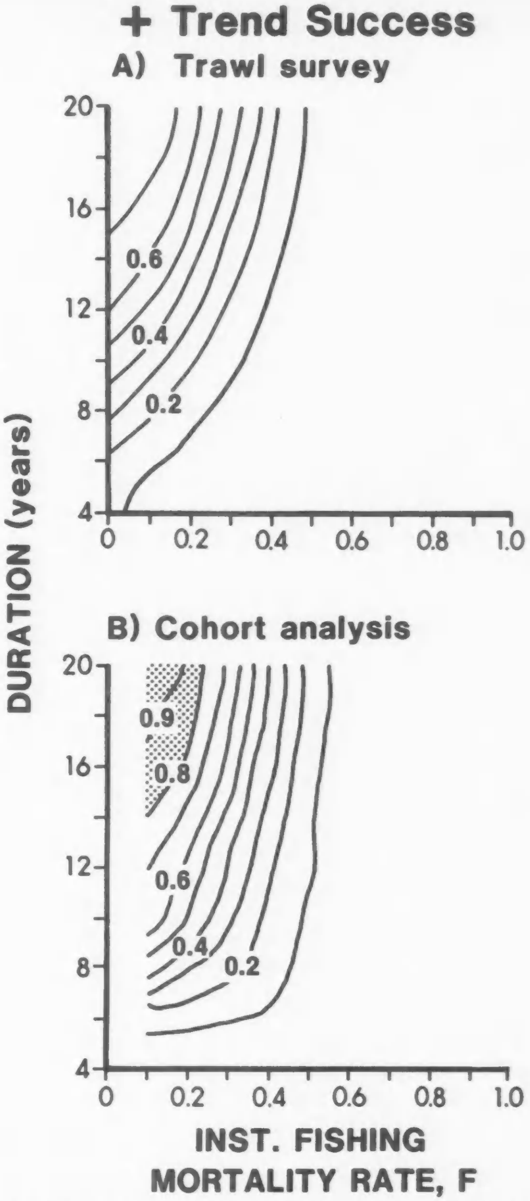

FIG. 6. Isopleths of probability that (A) trawl survey or (B) cohort analysis will meet management objective 1 for various combinations of instantaneous annual fishing mortality rates and durations of experiments in years. The stippled region encompasses those combinations which give a probability ≥0.8.

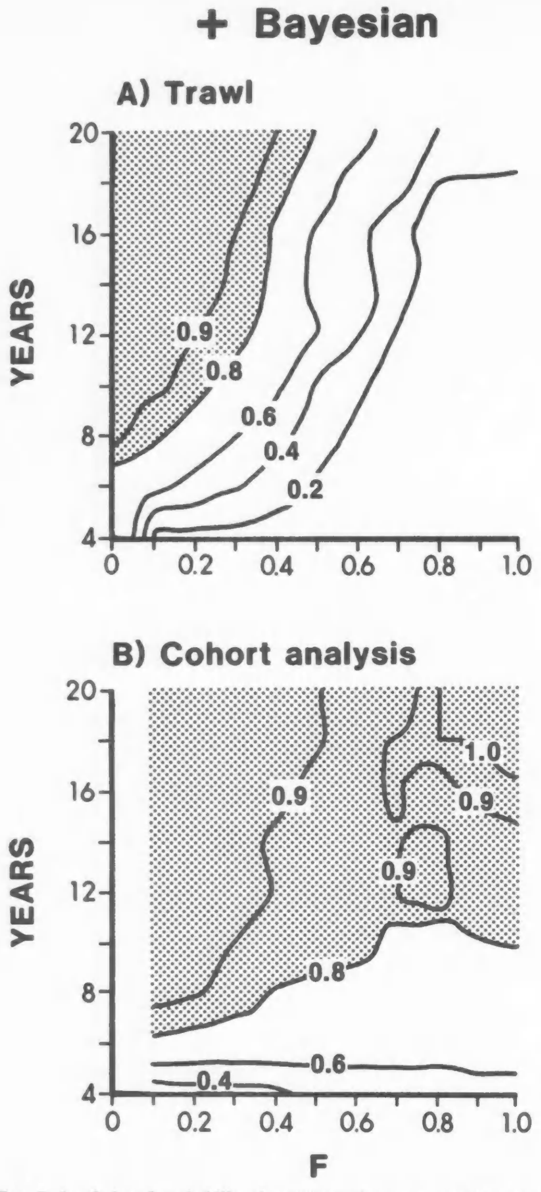

FIG. 7. Isopleths of probability that (A) trawl survey or (B) cohort analysis will meet management objective 2 for various combinations of instantaneous annual fishing mortality rates and durations of experiments in years. These are isopleths of power for detecting *increases* in abundance.

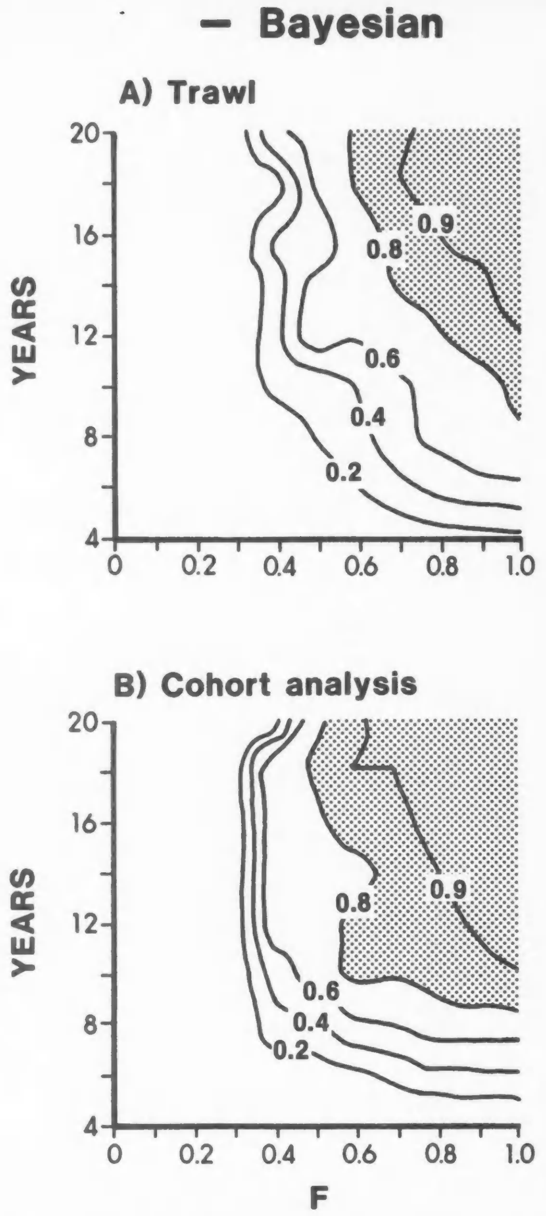

FIG. 8. Same as Fig. 7 but for management objective 3. These are isopleths of power for detecting *decreases* in abundance.

were used, at least 7 yr were required with no fishing but the necessary duration increased rapidly with increased fishing mortality rate (Fig. 7A).

The isopleths in Fig. 7B which refer to cohort analysis are not the same general shape as those in Fig. 7A because of a nonlinear interaction between the precision of cohort analysis and the probability that true trends will be positive; these two factors peak in opposite corners of the graph. Cohort analysis is most precise when *F* is large and the experiment is long (Pope 1972), so that the few positive trends that do occur under these conditions are correctly estimated. In contrast, a long experiment at low *F* is more likely to generate a steeper increasing true trend which does not require as precise a cohort analysis to be detected correctly. This asymmetric property of cohort analysis does not apply to trawl surveys (Fig. 7A); in situations where positive trends were weak or unlikely, the trawl survey did not identify them correctly.

Objective 3

This objective aimed to achieve a high probability that an observed significant *decrease* in estimated recruitment resulted from a significant *decrease* in true recruitment. Bayesian probabilities for this decreasing trend were acceptably high ($P \geq 0.8$) for cohort analysis only when $F > 0.4$ and more than 8 yr of data were available (Fig. 8B). Trawl surveys required at least 9 yr and high F values (Fig. 8A).

Discussion

We conclude that under most fishing plans that are feasible, there will be an unacceptably low probability of meeting management objective 1, 2, or 3, i.e. power will be low. Economic and political constraints will probably prevent managers from carrying out monitoring or experimental fishing programs at a constant F for long enough to achieve power ≥0.8. For example, in the case of objective 1, at least 14 yr are needed (Fig. 6B). Unless long-range funding methods and commercial fishing goals change, managers will most likely be unable to hold F constant for very long and thus they will be operating in the shorter-duration sections of our isopleth diagrams. The duration of resulting data series will usually be short (5–10 yr) and power will usually be less than 0.6 (Fig. 6B, 7B, 8B). Changes in fishing mortality imposed during an experiment must be avoided to prevent confounding of results. Ironically, even if longer programs were to become feasible, time trends in environmental driving variables would become more likely to confound interpretation of the results of stock assessments. Thus, these factors combine to reduce the probability that we can in practice correctly estimate changes in fish population abundances with trawl surveys or cohort analysis.

There was a high probability of an estimated trend in recruitment correctly reflecting a true trend (objectives 2 and 3) if the stock was subjected to either high or low fishing mortalities (Fig. 7 and 8). At low F, cohort analysis generally performed better than the trawl survey, most notably when estimates resulted in an increasing trend in abundance (Fig. 7). The power of the trawl survey could be increased if the sampling variability was reduced. When the model was run with σ_X halved to 0.2, power increased by 5–10%. However, a fourfold increase in sampling effort would be required for this small increase in power.

The results here represent optimistic estimates of power because stock assessment procedures simulated here all had unbiased input parameters. Biased inputs of M and F_{last} to the cohort analysis (Pope 1972; Sims 1984) and declining catchability with increasing abundance in the trawl survey (Garrod 1977) are two likely situations which will reduce power below the values calculated here. In addition, these biases have the ability to generate artificial trends in estimated abundance which may lead to incorrect or potentially dangerous conclusions about the true trends.

Because of other constraints, only a subset of our fishing plans (F and duration) that had acceptably high power will be acceptable to fisheries managers. For example, average annual catches or population abundance at the end of an experiment with high fishing mortality might be unacceptably low, even if the experiment gave a high probability of correctly identifying a trend (cf. Allen and Kirkwood 1976). Decision analysis techniques could be used to quantify for managers the trade-offs among these variables (Walker et al. 1983; Rothschild and Heimbuch 1983; Healey 1984). These methods could also quantify management objectives other than the three simple ones used here.

Our main conclusion concerning the prevalence of low power is generalizable to other stocks and species only if those other situations have similar (1) management objectives, (2) natural variation in population processes, and (3) errors in stock assessment methods. Generalizations may not be possible, for example, if the magnitude or structural form of variance terms (additive vs. multiplicative) is significantly different.

In spite of these restrictions on generalizations, other authors have also shown that statistical power is low in diverse fisheries situations. Allen and Kirkwood (1976) assumed that indices of whale abundance typically have CVs around 0.2. They estimated that to obtain a probability ≥0.8 of correctly concluding that abundance was significantly changing over time, at least 8 sample yr are required if the true rate of change is 10%/yr, and 13 yr are needed for a 5% rate of change. Allen and Kirkwood noted the practical difficulties of such lengthy experimental fishing situations. A more extensive review by de la Mare (1984) showed that the CV of whale abundance indices is more typically in the range 0.2–0.5. He calculated statistical power for detecting significant population trends using a variety of assumptions, and results were more pessimistic than those of Allen and Kirkwood. In all of these marine mammal examples, a simplified population model was used which assumed a constant rate of population change in abundance and that variation in abundance indices were due to sampling error alone. Power would be expected to be lower for marine fishes because of the relatively high interannual natural variation in population recruits (Hennemuth et al. 1980). Peterman and Routledge (1983) calculated statistical power for several potential experimental manipulations of Oregon coho salmon (*Oncorhynchus kisutch*). These experiments were designed to test whether marine survival was density dependent. Only a small subset of potential manipulations had power ≥0.8; large increases in production of juvenile salmon were needed for each of 5–10 yr.

We suggest that because many fisheries management programs are likely to be of low power, research programs, new regulations, and analysis of existing data should all be subject to power analysis. In most situations, regardless of the specific objective, it will not be possible to calculate power with an empirically based model of the managed population as we have done here. However, power can be approximated if sampling and abundance variances can be estimated (Cohen 1977; Toft and Shea 1983; Rotenberry and Wiens 1985). A proposed program that had low power would inform the manager that little confidence should be placed in its results, and that the program should be redesigned.

New Management Strategy

It has been shown in a variety of situations that methods of estimating time trends in abundance generally have unacceptably low probabilities of being correct (Allen and Kirkwood 1976; de la Mare 1984; this paper). One could then draw the conclusion that much more effort should be put into devising better indices of abundance (de la Mare 1984). However, even if it is feasible to develop adequately precise and accurate methods, their development is far in the future. In the interim, we propose that there should be a change in the way that current methods are used by management agencies.

Currently, scientists who estimate time trends in abundance of recruits (while monitoring intensively fished stocks, for example) assume that the null hypothesis (H_0) of no time trend is correct, until sufficient data are available to reject H_0. But we have shown here for English sole that many years (at least 5–10) are required at a constant F before sufficient data exist to have a test with high statistical power. This long period is caused by the large natural variability in population processes (e.g. Garrod 1983) and errors inherent in stock assessment methods. This required period has often been so long that stocks collapsed from recruitment overfishing before a decrease in recruitment was firmly shown (Saetersdal 1980). Numerous examples exist of collapsed clupeid stocks which may fit this situation (Murphy 1977): for example, Norwegian spring spawning herring, *Clupea harengus harengus*, Hokkaido–Sakhalin herring, *Clupea pallasii*, and the Southwest African/Namibian pilchard, *Sardinops ocellata* (Butterworth 1983). It therefore seems logical to recognize the limitations in our stock assessment methods and to propose a different way of dealing with time trends in harvested marine fish species.

We propose that the null hypothesis should be that abundance of recruits is decreasing at some specified rate, instead of assuming H_0: slope = 0. This new H_0 recognizes that there are strong economic capitalization processes at work in most fisheries that will lead to increased fishing mortality as time progresses (e.g. Paulik 1971). The examples of collapsed clupeid stocks mentioned above demonstrate that these economic forces frequently lead to recruitment overfishing before scientists are able to document that decrease and to convince management to take sufficient action (Saetersdal 1980). This inevitable increase in fishing power combined with the lag in perceiving the resulting decrease in recruitment leads to the second part of our new strategy. We would require that the H_0 of a decreasing trend in abundance of recruits be rejected before permitting an increase in F. In other words, we must be able to show that recruitment is significantly increasing or shows no significant time trend before fishing mortality is permitted to increase. To ensure impartiality, we propose that a joint industry–management agency team be responsible for the statistical analyses. Such analyses must be done for different assumed slopes of H_0 (e.g. 5, 10, 15% decrease per year) because the correct value will not be known.

The resulting management strategy would be very conservative. It essentially "assumes the worst until proven otherwise," which is in sharp contrast with the current approach, "assume no change until proven otherwise." An example of the current strategy is the International Whaling Commission's (1983) new management procedure which allows continued harvesting of a whale stock in the absence of any positive evidence that it is declining (cited by de la Mare 1984). We feel justified in "assuming the worst" in our proposed strategy because drastic declines in abundance have been very common. The history of fisheries management is not good — numerous fish stocks have collapsed, whether through natural environmental causes, overfishing, or a combination of the two. The best stock assessment methods have inherent errors, and fish stocks are naturally quite variable. Until present methods of assessment are radically improved, an extremely conservative approach to fisheries management, such as ours, may be advisable. Such a strategy will be difficult to implement and there will be numerous complications, but we must begin to explore alternatives to current methods. Simulated examples of the application of our proposed management strategy will be presented in a separate paper.

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Fish Catches and Long-Term Tidal Cycles in Northwest Atlantic Fisheries: A Nonlinear Regression Approach

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Selected long-term fisheries catch data from the New England – Fundy area and the Grand Banks were examined for concordance between changes in fish catches and the 18.6-yr nodal cycle of the tides using a nonlinear regression model. Significant positive correlations were found for Atlantic cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*), alewife (*Alosa pseudoharengus*), Atlantic herring (*Clupea harengus harengus*), and scallop (*Placopecten magellanicus*), with lag times that are biologically appropriate for the time from hatching to recruitment into the fishery. A significant negative correlation with the nodal cycle was evident for Atlantic menhaden (*Brevoortia tyrannus*), for which this area constitutes the most northerly part of its range. Cod catches on the Grand Banks showed no correlation with the nodal cycle. It is suggested that the correlations between the nodal cycle and the changes in fish catches are caused by correlated changes either in sea surface temperature or in productivity resulting from changes in the degree of vertical mixing.

À partir de longues séries de données sélectionnées sur les prises dans la région de la Nouvelle-Angleterre et de la baie de Fundy et celle des Grands Bancs de Terre-Neuve, on a cherché s'il y avait une concordance entre les changements dans les prises et le cycle nodal de 18,6 a des marées, en appliquant un modèle de régression non linéaire. On a observé des corrélations positives significatives pour la morue de l'Atlantique (*Gadus morhua*), l'aiglefin (*Melanogrammus aeglefinus*), le gaspareau (*Alosa pseudoharengus*), le hareng de l'Atlantique (*Clupea harengus harengus*) et le pétoncle (*Placopecten magellanicus*), avec des décalages biologiquement appropriés pour le temps de l'éclosion au recrutement. Par ailleurs, on a mis en évidence une corrélation négative significative avec le cycle nodal dans le cas de l'alose tyran (*Brevoortia tyrannus*) qui, dans ce secteur, se trouve dans la partie la plus au nord de son aire de distribution. On n'a établi aucune corrélation entre les prises de morue sur les Grands Bancs de Terre-Neuve et le cycle nodal. On émet l'hypothèse suivante: les corrélations entre le cycle nodal et les variations des prises seraient attribuables à des changements corrélés de la température à la surface de la mer ou de la productivité qui découleraient des changements du degré de mélange vertical.

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Many fisheries scientists assume that long-term fluctuations in fisheries yields are caused by low-frequency (multiyear) periodic changes in environmental variables. Long-term cycles that might be implicated include the (more or less) 11-yr sunspot cycle, the "double sunspot cycle" of 22 yr, the nodal tidal cycle of 18.6 yr, and the terrestrial "ecological cycle" of about 10 yr (cf. Bulmer 1974). Examinations of fish catch data have shown correlations with river discharge (Sutcliffe 1972, 1973), wind-induced circulation (Carruthers 1951; Chase 1955), residual drift (Redfield 1939; Colton and Temple 1961), water stability and eddies (Iselin 1939), large-scale weather systems (Dickson and Lee 1972; Iles 1973), major physical oceanographic events (Koslow 1984; Sinclair et al. 1985), and temperature (Templeman and Fleming 1953; Taylor et al. 1957; Martin and Kohler 1965; Flowers and Saila 1972; Dow 1977; Boudreault et al. 1977; Caddy 1979; Anthony and Fogarty 1985; and others). Each of these variables is undoubtedly important in determining mortality and/or growth rates of a fish population at one time or another.

Of these environmental variables, temperature exerts a most profound biological influence. Within a normal range of tolerance for a given species, growth and feeding rates are positively correlated with temperature. Larval survival is commonly the critical element in year-class strength, and since higher growth rates are associated with shortening of the vulnerable larval phase, higher temperatures would be expected to favour larval survival and hence enhance year-class strength. Anthony and Fogarty (1985) reported a strong positive relationship between temperature and yield of Atlantic herring (*Clupea* *harengus harengus*) in the Gulf of Maine, and Sutcliffe et al. (1977) found positive correlations between sea surface temperatures (SST) and Gulf of Maine catches of alewife (*Alosa pseudoharengus*), butterfish (*Peprilus triacanthus*), Atlantic herring, Atlantic menhaden (*Brevoortia tyrannus*), silver hake (*Merluccius bilinearis*), hard-shell clams (*Mercenaria mercenaria*) and scallops (*Magellanicus pecten*). In each case, lag times were found between peak temperatures and peak catches that were more or less appropriate for the time taken to develop from larval stages to fishable size. Significant negative correlations, however, were found between SST and yields of Atlantic cod (*Gadus morhua*), redfish (*Sebastes marinus*), striped bass (*Morone saxatilis*), yellowtail flounder (*Limanda ferruginea*), and soft-shell clams (*Mya arenaria*). With appropriate lag times, catches of these species were higher following years of lower SST. Similar analyses of data for Atlantic halibut (*Hippoglossus hippoglossus*), red hake (*Urophycis chuss*), cusk (*Brosme brosme*), and pollock (*Pollachius virens*) produced equivocal results. Sutcliffe et al. (1977) did not identify the causes of positive or negative correlations in the data except to note that most of the species showing negative correlations with SST were near the southern limits of their geographic range. It is implicit that not all species respond to temperature in the same way and that some other mechanism may explain negative correlations between temperature and fish catches.

An important environmental variable that influences SST is tidal mixing. Loder and Garrett (1978) have shown that SST in east coast locations varies over an 18- to 19-yr cycle in concordance with the 18.6-yr nodal cycle; the tidal modulation causes a variation of ±5% of the average temperature range from top to bottom of the water column. The effect may be masked by variations in river outflow, particularly from the St. John and St. Lawrence rivers.

Tidal mixing also promotes nutrient recirculation to surface waters, stimulating primary production. Consequently, an increase in fish catch following the peak years of the nodal cycle might equally result from food chain effects of increased productivity (Campbell and Wroblewski 1986).

On the basis of the demonstrated negative relationship between nodal cycle and temperature, and the assumed positive relationship with productivity, we hypothesized that fish stocks of the Gulf of Maine – Bay of Fundy area should show correlations with the nodal cycle. The direction of the correlation should depend upon the precise mechanism by which temperature or food supply affects differential survival and recruitment: positive correlations with temperature might indicate a species near the northern limit of its range; negative correlations would result if larval survival was enhanced either by lower temperatures or the higher productivity associated with peaks of the nodal cycle. In this study we examined a few of the longer data sets available for the Gulf of Maine – Bay of Fundy system to see whether any correlation exists with the nodal cycle. We also examined some data from the Grand Banks, where the tidal signal is much reduced by comparison with other oceanographic processes.

Procedures

Many of the long-term data sets that were used by Sutcliffe et al. (1977) were obtained from the Marine Ecology Laboratory, Bedford Institute of Oceanography. Where possible, these files were updated with data from years since 1971–74 at which Sutcliffe's data files ended. In addition, several new data sets were compiled from the files at Saint Andrews Biological Station (see Table 1).

TABLE. 1. Fisheries data sets and the lags by which the 18.61-yr nodal cycle was offset. A negative year in the lag column indicates that the nodal cycle lags the fish catch; otherwise the fish catch lags the nodal cycle. NE = New England; PQ = Province of Quebec; SW Nova = southwest Nova Scotia; Ch. Co. = Charlotte County, New Brunswick; area 5 = ICNAF area 5 (Gulf of Maine); area 3 = ICNAF area 3 (Newfoundland). Data series and sources: M = Marine Ecology Laboratory; S = St. Andrews Biological Station.

Species	Source	Length (yr)	Lag (yr)
Alewife, NE	M	54	0
Butterfish, NE	M	54	1
Atlantic cod, area 5	M	89	5
Atlantic cod, area 3	M	75	5
Atlantic cod, PQ[a]	M	46	
Haddock, area 5	M	89	5
Atlantic halibut, area 5	M	85	5
Atlantic herring, NE[a]	M	53	
Lobster, SW Nova	S	55	0
Mackerel, NE[a]	M	53	
Atlantic menhaden, NE	M	49	−3
Pollock, NE	M	54	0
Redfish, area 5	M	43	−6
Sardines, Ch. Co	S	104	2
Scallops, Digby[a]	S	62	
Scallops, NE	M	50	1
Striped bass, NE[a]	M	52	
Silver, hake, NE	M	44	1
Yellowtail flounder, NE[a]	M	40	

[a]Data set was not analyzed (see text).

Annual catch data for species that had at least 40 yr of continuous record were plotted and visually inspected for evidence of low-frequency periodicity. A synthetic nodal cycle with an 18.61-yr period was computed for the years 1860 to 1984 for the purpose of examining the relationship between fish catch variations and the 18.6-yr nodal cycle.

For the purposes of the modelling, the values of the lag by which to offset the nodal cycle were selected in the following manner.

The data sets were smoothed using a seven-point Cartwright low-pass filter, having a cut-off of about 1 cycle in 4 yr. This filter effectively removes the high-frequency portion of the data and greatly aids in the visual correlation of the fish catch data and the nodal cycle. The cross-correlation function (CCF) between the nodal cycle and each catch data set was then computed. The lag at which the maximum value of this correlation occurred was the one chosen for further analysis. For some of the data sets, the CCF did not have an obvious peak; these data were not further analyzed. The CCF's for the species for which there was a significant peak are shown in Fig. 1.

The fisheries data exhibit a marked persistence in that data points tend to remain on one side or the other of the mean for several successive years. A consequence of this is that the data are highly autocorrelated, and thus, all observations in the time series are not independent. Previous researchers accounted for this by computing the significance level after the effective number of independent observations (n^*) and the "reduced degrees of freedom" ($n^* - 2$) had been determined. This

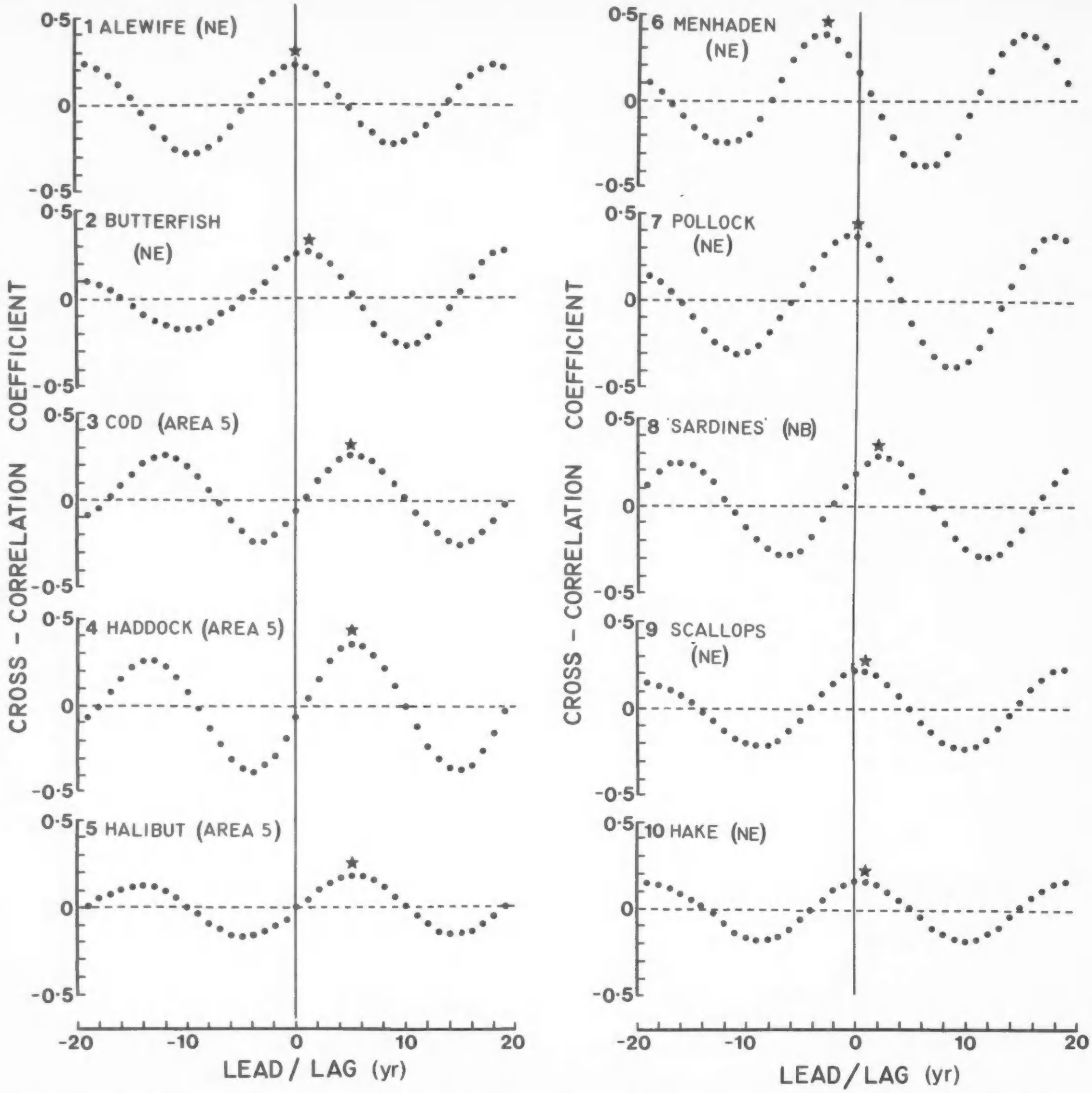

FIG. 1. Variation in cross-correlation coefficients between fish catch and the nodal cycle for 10 stocks of the Bay of Fundy – Gulf of Maine – Georges Bank area. NE = New England; NB = New Brunswick (Charlotte County only); area 5 = ICNAF area 5.

problem is discussed in more detail in Sutcliffe et al. (1976). We did not use this approach because the assumptions made in the determination of n^* are essentially arbitrary. In addition, the nodal cycle is a deterministic signal. Our approach is instead a nonlinear regression model.

To explore the possible relationship between the variable y_t = catch and x_t = appropriately lagged nodal value, we considered the regression model

$$(1) \quad \begin{cases} y_t = \beta_0 + \beta_1 x_t + e_t \\ e_t = \rho e_{t-1} + a_t \qquad t = 1, 2, \ldots, n. \end{cases}$$

Here, the regression errors e_t follow a stationary first-order autoregressive process, with independent identically distributed error terms $\{a_t\}$ with mean 0 and variance σ^2. Cochrane and Orcutt (1949) indicated three important reasons why a regression model may have nonindependent errors $\{e_t\}$: (i) faulty choice of the form of the regression model, (ii) omission of important variables from the model, and (iii) use of incorrect variables or poor data.

While the main purpose of this study was to investigate the relationship between the fisheries and the nodal tidal cycle, it is clear that in order to construct an appropriate model for "catch," many other variables should be considered as predictors. Further, the values of the response variable "catch" may not be totally reliable. Thus, it would appear that in this case at least two of the reasons given by Cochrane and Orcutt (1949) are in effect. This model with order one autoregressive errors is a special case of the model with stationary mixed autoregressive moving average errors of order p and q, respectively, considered in Pierce (1971a):

$$(2) \quad \begin{cases} y_t = \sum_{i=1}^{k} \beta_i x_{it} + e_t \\ \phi(B)e_t = \theta(B)a_t \end{cases}$$

with

$$Be_t \; = e_{t-1}$$

(backward shift operator)

$$\phi(B) = (1 - \phi_1 B - \ldots - \phi_p B^p)$$

(autoregression operator)

$$\theta(B) = (1 - \theta_1 B - \ldots - \theta_q B^q)$$

(moving average operator)

and $\{a_t\}$ independent identically distributed with mean 0 and variance σ^2. A further generalization to nonstationary errors is considered in Tsay (1984). We note that the simple model we are considering can be written as

$$(3) \quad y_t - \rho y_{t-1} = \beta_0(1 - \rho) + \beta_1(x_t - \rho x_{t-1}) + a_t.$$

With $z_t = y_t - \rho y_{t-1}$, $w_t = x_t - \rho x_{t-1}$, $\beta_0' = \beta_0(1 - \rho)$, this becomes a simple linear regression with independent errors:

$$(4) \quad z_t = \beta_0' + \beta_1 w_t + a_t \qquad t = 2, \ldots, n.$$

(Note the reduction of the sample size from n to $n - 1$.) Similarly, the more general model may be transformed to a multiple linear regression.

Estimation of the Parameters

Rewriting the model in Pierce (1971a) as

$$\phi(B)y_t = \phi(B)\left(\sum_{i=1}^{k} \beta_i x_{it}\right) + \phi(B)e_t,$$

we have

$$y_t - \sum_{j=1}^{p} \phi_j y_{t-j} = \sum_{i=1}^{k} \beta_i x_{it} - \sum_{j=1}^{p} \sum_{i=1}^{k} \beta_i \phi_j x_{i,t-j} + a_t - \sum_{k=1}^{q} \theta_k a_{t-k}$$

giving

$$(5) \quad a_t = \sum_{k=1}^{q} \theta_k a_{t-k} - \sum_{j=0}^{p} \phi_j y_{t-j} + \sum_{i=1}^{k} \sum_{j=0}^{p} \beta_i \phi_j x_{i,t-j}$$

where $\phi_0 = -1$.

With appropriate starting values $a_{-q+1}, \ldots, a_{-1}, a_0, y_{-p+1}, \ldots, y_0, x_{i,-p+1}, \ldots, x_{i0}$ specified, the estimates of $(\beta_1, \ldots, \beta_k, \phi_1, \ldots, \phi_p, \theta_1, \ldots, \theta_q, \sigma^2)$ are those which minimize the sum of squared residuals, $\Sigma \hat{a}_t^2$. Such minimization must be effected by some *nonlinear* least squares estimation method in order to estimate simultaneously all the parameters. This method of Pierce (1971a) gives approximate maximum likelihood estimates and is an extension of the "conditional sum of squares approach" in Box and Jenkins (1970). An alternative approach is found in Harvey and Phillips (1979) where the maximum likelihood estimates are calculated through the use of Kalman filter techniques.

For the model given in (1) the method of Pierce (1971a) reduces to finding the values $\hat{\beta}_0$, $\hat{\beta}_1$, $\hat{\rho}$, and $\hat{\sigma}^2$ for which

$$(6) \quad \sum_{t=2}^{n} \hat{a}_t^2 = \sum_{t=2}^{n} ((y_t - \hat{\rho} y_{t-1}) - \hat{\beta}_0(1 - \hat{\rho}) + \hat{\beta}_1(x_t - \hat{\rho} x_{t-1}))^2$$

is a minimum. Notice that the residuals $\hat{a}_t$ are the differences between the "observed" value y_t, and the "fitted" value $(\hat{\beta}_0 + \hat{\beta}_1 x_t) + \hat{\rho}(y_{t-1} - (\hat{\beta}_0 + \hat{\beta}_1 x_{t-1}))$. An algorithm for calculating these estimates is given in Hildreth and Lu (1960). This consists of scanning for possible values of $\hat{\rho}$ in steps from -1 to 1. For each such value of $\hat{\rho}$, say $\hat{\rho}_0$, the values $\hat{\beta}_0$ and $\hat{\beta}_1$ are the least squares estimates of the regression (4) with $\rho = \hat{\rho}_0$, and the resulting residual sum of squares is the corresponding value of $\Sigma \hat{a}_t^2$. The search continues until the value $\hat{\rho}$ is found for which (6) is a minimum.

When the distribution of the $\{a_t\}$ is normal, Hildreth (1969) has shown that the maximum likelihood estimator of the parameters (β_0, β_1), ρ, and σ^2 has asymptotically (as $n \rightarrow \infty$) a multivariate normal distribution with mean equal to the parameters and a covariance matrix such that $(\hat{\beta}_0, \hat{\beta}_1)$, $\hat{\rho}$, and $\hat{\sigma}^2$ are asymptotically independent. Pierce (1971a) showed that this asymptotic result holds even when the $\{a_t\}$ are not normally distributed.

Methods of Inference

To determine the importance of the contribution of the nodal cycle to the catch, we are interested in testing H_0: $\beta_1 = 0$ versus H_1: $\beta_1 > 0$. A rejection of H_0 indicates that there is a linear association. Inferences on the regression parameters depend on the validity of the assumed error model, and this must also be examined. Under the normal model, if ρ were a known constant, then $\hat{\beta}_1$ would have a normal distribution with mean β_1, and variance $\sigma^2(\text{ss}_w)^{-1}$ where ss_w is the corrected sum of squares:

$$\text{ss}_w = \sum_{t=2}^{n} w_t^2 - \frac{1}{n-1}\left(\sum_{t=2}^{n} w_t\right)^2.$$

This asymptotic distribution is valid even when the $\{a_t\}$ are nonnormal, and in addition, if these error distributions are symmetrical, both $\hat{\rho}$ and $\hat{\sigma}^2$ are asymptotically independent of $\hat{\beta}_1$. Thus for n large,

$$(7) \quad T = \frac{\hat{\beta}_1 - \beta_1}{\hat{\sigma}\sqrt{\sum_{t=2}^{n} \hat{w}_t^2 - \frac{1}{n-1}\left(\sum_{t=2}^{n} \hat{w}_t\right)^2}},$$

where $\hat{w}_t = x_t - \hat{\rho} x_{t-1}$, has approximately a standard normal distribution. A limited Monte Carlo study in Pierce (1971a) indicates that even for moderate sample sizes the exact and approximating distributions are not very far apart. A somewhat more conservative approximation would be a t distribution with $(n - 1) - 2 = n - 3$ degrees of freedom.

To examine the validity of the error model, we rely on results of Pierce (1971b). For n large, the first s sample autocorrelations, r_i, $i = 1, \ldots, s$, of the residuals calculated from a least-squares fit of a regression model with autoregressive moving average errors follow a multivariate normal distribution with a covariate structure such that

$$n \sum_{k=1}^{s} \hat{r}_k^2$$

TABLE 2. Estimated model parameters and the significance of the linear relationship between fisheries data and the 18.61-yr nodal cycle. The lags used are given in Table 1. NE = New England; Ch. Co. = Charlotte County, New Brunswick; area 5 = ICNAF area 5.

Species	$\hat{\rho}$	$\hat{\beta}_0$	$\hat{\beta}_1$	T	Significance
Alewife, NE	0.70	−39.667	42.732	1.75	0.025–0.05
Butterfish, NE	0.78	−9.854	11.454	1.48	0.05–0.1
Atlantic cod, area 5	0.83	−127.873	163.062	2.22	0.015–0.02
Haddock, area 5	0.81	−336.659	379.794	2.52	0.005–0.01
Atlantic halibut, area 5	0.76	−1955.250	2236.420	1.45	0.05–0.1
Atlantic menhaden, NE	0.78	−240.185	247.657	2.82	0.003–0.005
Pollock, NE	0.79	−44.107	55.726	1.68	0.05–0.1
Sardines, Ch. Co.	0.40	−158.457	200.726	2.06	0.02–0.025
Scallops, NE	0.93	−22.672	29.011	1.93	0.025–0.05
Silver hake, NE	0.87	−73.653	104.327	0.93	0.15–0.2

has approximately a χ^2 distribution with df = $s - 1$. This leads to a "portmanteau" test of lack of fit (which was applied) and parallels the result for time series in Box and Pierce (1970). Gross deviations from fit can also be detected by noting that the asymptotic variances of the r_k's are all less than $1/n$. Finally, the residuals were tested for first-order autoregression through the Durbin–Watson test.

Results

In all data series analyzed (Table 1), the search for ρ was done at first in 0.1-unit steps, and when the approximate value of ρ was identified, in 0.005-unit steps around this value. The catch data were regressed against nodal values with various leads or lags as below:

$$\text{Fish catch } (t) = \beta_0 + \beta_1 \text{ nodal } (t \pm \text{lag}) + e_t.$$

Estimated model parameters and significance test results are given in Table 2.

Of 12 stocks analyzed from the region of the Gulf of Maine (ICNAF area 5) (Table 1), three (Atlantic cod, Atlantic halibut, and haddock) comprise the most important commercial finfish of the region. For Atlantic cod and haddock, positive linear relationships were found between the annual catch records and the nodal cycle, with a 5-yr time lag between the peak of the nodal cycle and the peak catch. These lags (computed from the CCF) correspond well with the average time from hatching to recruitment into the fishery. Menhaden catches are significantly related to the nodal cycle but with a 3-yr lead with respect to the nodal peak. Of the remaining stocks, records for alewife, Atlantic herring, and scallops also exhibit statistically significant relationships with the nodal cycle, with biologically appropriate lag times.

Other species (Atlantic halibut, butterfish, and pollock) show relationships with the nodal cycle which are almost acceptable at the 5% level. Further, lobster, redfish, and area 3 cod stocks were found not to be significantly linearly related to the nodal cycle.

Both lobster and redfish are long-lived, bottom-dwelling species, individuals of which may contribute to recruitment over many years. Redfish do not enter the fishery in abundance until 12–14 yr of age (compared with 7–9 yr for lobsters) and are caught until at least age 25. Natural mortality rates are extremely low (Mayo 1980). Furthermore, the dynamics of the Gulf of Maine – Georges Bank redfish fishery have changed considerably over the period of record. All of these factors may well mask a weak correlation with the nodal cycle if such exists.

It is useful to investigate how the significance of the linear component of the relationship between a fish species and the nodal cycle varies as a function of the choice of lag. Using the haddock catch data as an example, the significance was calculated for lags ranging from 2 to 7 yr. Table 3 clearly shows that the significance peaks at the lags of 4–5 yr.

Figure 2 demonstrates the relationships between some of the longer data sets and the nodal cycle. To aid visual comparisons, Atlantic cod catches for Newfoundland, which are uncorrelated with the tides, are also shown (Fig. 2A). Filtered (Fig. 2F) and unfiltered (Fig. 2G). records of "sardine" (2-yr-old Atlantic herring) catches in weirs of Charlotte Co. (N.B.) illustrate the effect of the low-pass filter on the data. Vertical scales vary from panel to panel and have been omitted in order to emphasize the pattern relationships with tidal cycles in catch data of greatly different volume.

Another means of exploring the reality of these correlations is to compare different stocks of the same species, particularly where one stock might be expected to show no relation to the nodal cycle or be more strongly influenced by some other environmental variable. We compared the Atlantic cod catches for Newfoundland (ICNAF area 3) and the Gulf of Maine (ICNAF area 5) on the assumption that the Grand Banks area would receive a much reduced absolute tidal signal that should be completely masked by other environmental variables. Figure 3 presents spectral plots of the variance for the two stocks in relation to cycle length. As expected, there was no statistical significance between the catch data and the nodal cycle for area 3 Atlantic cod; a greater percentage of the variance is associated

TABLE 3. Estimated model parameters and variation of the significance of the linear relationship between the haddock catch and the nodal cycle as a function of the lag (years) of the haddock catch after the nodal cycle.

Lag (yr)	$\hat{\rho}$	$\hat{\beta}_0$	$\hat{\beta}_1$	T	Significance level
2	0.84	−137.5	180.5	1.13	0.10–0.15
3	0.83	−228.5	271.9	1.74	0.025–0.05
4	0.82	−325.4	369.6	2.46	0.008–0.01
5	0.81	−351.9	394.7	2.52	0.005–0.08
6	0.81	−336.7	379.8	2.39	0.008–0.01
7	0.82	−247.7	292.4	1.86	0.025–0.05

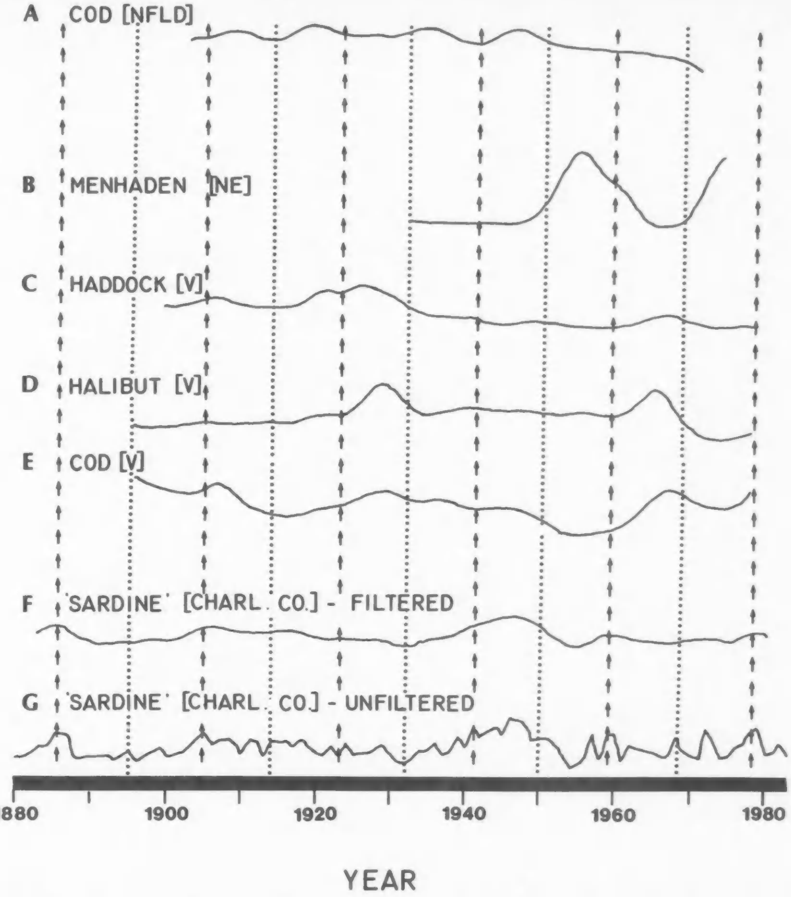

FIG. 2. Commercial landings for five fish species in relation to the 18.6-yr nodal cycle. (A) Atlantic cod landings in Newfoundland (ICNAF area 3); (B) Atlantic menhaden landings in New England; (C) haddock landings for ICNAF area 5; (D) Atlantic halibut landings for ICNAF area 5; (E) Atlantic cod landings for ICNAF area 5; (F and G) "sardine" (i.e. 2-yr-old Atlantic herring) landings in Charlotte Co., New Brunswick. Figure 2G presents unfiltered catch data for comparison with the filtered data shown in Fig. 2F. Arrows and dotted lines indicate years of peak (i.e. highest tidal range) and trough (lowest range) in the nodal cycle.

with a repetitive cycle of about 11 yr duration. In contrast, the greatest variance for Gulf of Maine landings is correlated with a cycle of 18–19 yr. The spectral plots shown in Fig. 3 indicate that the variance of the landings for these two species at the nodal frequency is only about 5% for the area 3 Atlantic cod but about 23% for area 5 Atlantic cod.

Catch data for Atlantic menhaden exhibit a cycle that leads the nodal cycle by 3–4 yr. This is a southern species that appears in the Gulf of Maine in years of warmer SST (Bigelow and Schroeder 1953). It is recruited into the fishery at ages of 3–5 yr; thus, its peak appearance before the peak of the nodal cycle suggests that the species responds favorably to the warmer years associated with the trough of the nodal cycle. Sutcliffe et al. (1977) found this species to exhibit a highly significant correlation with SST with a lag of about 3 yr.

The fact that spawning individuals of this species have recently been found in the Bay of Fundy system (R. A. Rulifson, Institute for Coastal and Marine Research, East Carolina University, Greenville, NC, and K. Stokesbury, Acadia Centre for Estuarine Research, Acadia University, Wolfville, N.S., pers. comm.) suggests that significant changes may be occurring in the distribution and abundance of this species. Alternatively, periodic spawning in northerly waters may be an unrecognized feature of the low years of the nodal cycle.

Discussion

Our results indicate that a significant linear relationship exists between fisheries and catch data in the Bay of Fundy – Gulf of Maine – Georges Bank (FMG) area and variations in the strength of tidal mixing over the 18.61-yr nodal cycle. The same cannot be said of areas such as the Grand Banks, where the tidal signal is much less influential by comparison with other environmental variables.

It is surprising that the influence of such a cycle can be

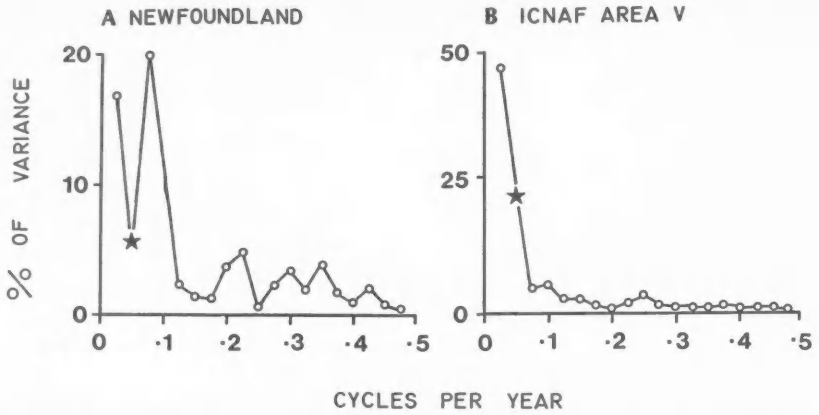

FIG. 3. Comparison of variance associated with cycles of varying length in Atlantic cod landing data for (A) Newfoundland (ICNAF area 3) and (B) the Gulf of Maine (ICNAF area 5). Note the different scales.

detected in raw catch figures because there are so many confounding influences upon the data. Apart from obvious changes in fishing effort, there have been important changes in fishing efficiency through improvements in ability to locate fish and in net construction and design. Moreover, the data are not necessarily "pure" in themselves: fishermen do not always report the size and location of catches with complete accuracy, and many fishing techniques capture several species together that are not always recorded separately. The introduction of foreign fishing fleets exacerbates the problem of accurate recording.

Our analytical approach involves established nonlinear regression techniques. Since the catch data are subject to uncertain errors and not all possible variables are being used, it is probable that the model errors are not independent. Consequently, a nonlinear regression is appropriate (Cochrane and Orcutt 1949). The method used is robust and avoids the arbitrary assumptions made in previous analyses such as those used by Sutcliffe et al. (1977).

It is apparent that not all species respond in the same way to the nodal cycle. Northern species seem to show a positive correlation with the nodal cycle and a negative relationship with temperature. Southern species do the reverse, while some are apparently unrelated to the cycle. Explanation of a given response depends upon the details of the life cycle and distribution of the species concerned.

The precise mechanism by which variations in tidal amplitude influence the fisheries in the FMG area is at present unknown. A clear negative correlation exists between tidal amplitude and SST (Loder and Garrett 1978) over the nodal cycle, and since temperature is such a potent biological variable, it may be that many species are responding directly to temperature variations. Using much shorter data sets, strong correlations have been found between catch data and sea temperatures in the New England area (e.g. Chase 1955; Dow 1964, 1977; Anthony and Fogarty 1985). It is equally probable, however, that the peaks of the nodal cycle correspond with increased primary production stimulated by nutrient circulation. It may be this greater food supply that is associated with higher larval survival, and hence greater catches some years later when these more numerous fish have reached catchable size. Another mechanism may be the influence of tidal amplitude on larval retention in estuaries. If greater retention within an estuary such as the Bay of Fundy leads to enhanced survival of fish larvae, years of low tidal range may well produce good year classes of those species whose larvae depend upon estuarine habitats. This might result in a negative correlation with the nodal cycle, and an apparent (possibly spurious) positive correlation with temperature. A given species might also be responding to the cycle indirectly, through oscillations of important food species or predators. Such an "ecological" effect would be difficult to distinguish from a direct response to the nodal cycle, although presumably the correlation would not be as high.

We conclude that some of the existing fisheries data indicate a clear influence of tidal amplitude changes over the nodal cycle on some fish stocks in the FMG area. For certain species of great commercial value the influence appears to be positive: fish catches rise after peaks of tidal mixing, the variation being somewhat greater than the 2.5–3.7% modulation of tidal amplitude. For other species such as Atlantic herring and Atlantic menhaden, the correlation with the tidal cycle is negative.

On the basis of these results, it seems probable that increasing tidal range by the construction of a tidal barrage in the upper Bay of Fundy (Greenberg 1984) will produce an increase (possibly proportional) in some of the fish yields in the FMG area and a decrease in some others. Firm predictions cannot be made, however, without a much better understanding of the relationship between tidal amplitude and productivity in these shallow waters.

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Contribution of Calcite to the Particle-Size Spectrum of Lake Michigan Seston and Its Interactions with the Plankton[1]

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Vanderploeg, H. A., B. J. Eadie, J. R. Liebig, S. J. Tarapchak, and R. M. Glover. 1987. Contribution of calcite to the particle-size spectrum of Lake Michigan seston and its interactions with the plankton. Can. J. Fish. Aquat. Sci. 44: 1898–1914.

We determined the contribution of calcite to the total particle-size spectrum of Lake Michigan seston during different seasons in 1978–83 and 1985, employing a novel Coulter counter method, to examine the intensity and ecological effects of calcite whitings that result from autogenic precipitation of calcite. The whitings were most intense during September, when 12–56% of the total particle volume was calcite. Overall, food web dynamics were more controlling of than controlled by whitings. Year-to-year variation in calcite concentration was probably caused by predation-controlled variation in primary production, which drives calcite precipitation through CO_2 uptake. Feeding rate of cladocerans in Lake Michigan is slightly reduced (16%) by calcite, but that of copepods is not. Light extinction is only slightly increased. Coprecipitation and sinking of P with calcite is probably a minor factor in regulating P concentration in the epilimnion of Lake Michigan. Removal of nutrients is enhanced by increased sinking rates caused by inclusion of calcite in fecal pellets and matter. Although all of the effects of calcite on plankton dynamics appear to be subtle in Lake Michigan, large effects can be expected for lakes of greater calcium hardness and eutrophy.

Nous avons déterminé la contribution de la calcite au spectre total de taille des particules du seston dans le lac Michigan durant différentes saisons de 1978 à 1983 et en 1985 à l'aide de nouvelles méthodes de mesure utilisant un compteur de Coulter. Nous voulions examiner l'intensité et les effets écologiques des suspensions de carbonate de calcium provenant de la précipitation autogène de la calcite. Ces suspensions étaient les plus intenses en septembre, la calcite constituant alors de 12 à 56 % du volume total des particules. Dans l'ensemble, la dynamique des réseaux trophiques régularisait davantage les suspensions de carbonate de calcium qu'èlle ne l'était par celles-ci. Les variations de la concentration de la calcite entre les années sont probablement dues à des variations de la productiòn primaire reliées à la prédation; la production primaire, par l'absorption de CO_2, détermine la précipitation de la calcite. L'alimentation des cladocères dans le lac Michigan est légèrement réduite (16 %) par la calcite, mais pas celle des copépodes. L'atténuation de la lumière n'est que légèrement augmentée. La coprécipitation et la sédimentation du P avec la calcite jouent probablement un rôle mineur dans la régulation de la concentration du P dans l'épilimnion du lac Michigan. La présence de calcite dans les matières et pelotes fécales accélère leur sédimentation et, par conséquent, favorise une perte de substances nutritives. Même si tous les effets de la calcite sur la dynamique du plancton semblent ténus dans le lac Michigan, on peut s'attendre à des effets importants dans les lacs où la dureté calcique et l'eutrophie sont plus prononcées.

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During late summer, calcite crystals precipitate autogenically in Lake Michigan (Strong and Eadie 1978; Eadie 1980), causing the milky water, or "whitings" (Bathurst 1971), reported for Lake Michigan (Ayers et al. 1967; Strong and Eadie 1978). Satellite imagery has shown that these whitings occur in surface waters over most of the lake area. This precipitation occurs in response to lowered solubility of $CaCO_3$ caused by an increase in water temperature and by an increased activity of CO_3^{2-} resulting from CO_2 uptake (and pH increase) by phytoplankton. Calcite whitings appear to be particularly intense in September, when epilimnetic temperatures exceed 20°C and pH values are greater than 8.4.

The abundance and particle size of calcite had seldom been documented for lakes (e.g. Brunskill 1969; Weidemann et al. 1985). Moreover, the ecological consequences of calcite whitings to plankton dynamics are poorly understood. Previous studies on the effects of calcite crystals on plankton dynamics have emphasized the following (Koschel et al. 1983): (1) decreased availability of P caused by coprecipitation with calcite (e.g. Ostuke and Wetzel 1972) and subsequent sinking out of the water column; (2) light limitation of phytoplankton caused by increased turbidity from calcite (e.g. Minder 1923; Weidemann et al. 1985); (3) increased sedimentation of phytoplankton and bacteria due to their aggregation with calcite crystals (self-flocculation processes) (Koschel et al. 1983).

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Effects of calcite on zooplankton feeding and effects of zooplankton feeding on calcite crystals have not been explored. Scanning electron micrographs of the whiting particles in Lake Michigan showed that they are individual or aggregated calcite crystals ranging in size from approximately 1 to 70 μm (Strong and Eadie 1978). *Diaptomus*, the dominant copepod genus and a major herbivore in Lake Michigan, is a size-selective suspension feeder that feeds on seston within this size range (Vanderploeg and Scavia 1979; Vanderploeg 1981; Vanderploeg et al. 1984). Filter-feeding cladocerans also feed on particles over much of this size range (Gliwicz 1969; Porter et al. 1983). Copepods and cladocerans ingest inert particles (Brunskill 1969; Gliwicz 1969; Paffenhöfer 1972; Honjo and Roman 1978), which can cause reduced ingestion of food (Paffenhöfer 1972; Arruda et al. 1983; McCabe and O'Brien 1983) leading to lower growth rates and higher mortalities (Paffenhöfer 1972; McCabe and O'Brien 1983). The passive and active modes of feeding used by *Diaptomus* (Vanderploeg and Paffenhöfer 1985) imply that ingestion of calcite and noncalcarious seston will be influenced by amounts of calcite and other seston. Likewise, other ecological effects of calcite depend on not just amount but also size of calcite. For example, light scattering, the major mechanism of light attenuation by suspensoids like calcite (e.g. Kirk 1985), and calcite sinking rate, which affects removal rate of coprecipitated P, strongly depend on calcite size. Therefore, an evaluation of ecological effects of calcite depends on measuring the calcarious and noncalcarious contributions of natural particle-size spectra.

In this paper we present a Coulter counter method to determine the contribution of calcite to the total particle-size spectrum of lake water. Particular emphasis is given to method evaluation and verification because our methods represent a novel application of the Coulter counter. We document the contribution of calcite to the particle-size spectrum during different seasons and years. From knowledge of the calcite contribution, ecological effects were determined from laboratory experiments and by extrapolation from the work of others. We examined effects of calcite on the feeding of *Diaptomus* and *Daphnia* in experiments utilizing natural seston containing calcite and in experiments utilizing laboratory cultures of algae and laboratory-generated calcite. We determined effects of calcite on light scattering and on coprecipitation and removal of P from literature information and from knowledge of the calcite contribution. During the course of our study we discovered that great quantities of calcite were incorporated in zooplankton fecal pellets. We determined the degree to which this inclusion increased fecal pellet sinking rate and its potential effects on cycling of fecal-bound nutrients. A comprehensive picture of calcite and plankton interactions in Lake Michigan was sketched.

Verification of Coulter Method

Experimental Approach and Rationale

The Coulter counter method for measuring the contribution of calcite to the total particle-size spectrum of lake water depends on the premise that addition of dilute acid to lake water will dissolve the calcite crystals. The particle-size spectrum of calcite is estimated by subtracting the particle-size spectrum of the acid-treated sample from that of the untreated sample. For this method to work, the concentration of acid used must be high enough to dissolve the calcite crystals in a relatively short period, yet be low enough not to affect the particle-size spectrum of algae and other organic seston.

The difference between particle-size spectra for untreated and acid-treated lake water samples (after sufficient acid exposure time) should be an accurate measure of the calcite particle-size spectrum, assuming calcite is the only acid-labile component of the seston, because the difference between particle spectra of untreated and acid-treated samples is dependent only on the Coulter counter's ability to properly size calcite crystals. Calcite crystals, which usually are either rhombic or nearly round, will be very accurately sized, since the Coulter counter was calibrated with microspheres (e.g. Gregg and Steidly 1965; Kachel 1976; Vanderploeg 1981b). Also, the methods described here should provide reasonable estimates of particle size of the noncalcareous seston (algae and detrius) (Vanderploeg 1981b), which have more variable shapes. In freshwater, algae with calcareous tests are rare (e.g. Kelts and Hsu 1978); therefore, this potential error source in marine waters is not important for freshwater.

The method is verified by showing that the following conditions are satisfied. First, when calcite is not a component of seston, acid addition has negligible effect on the particle-size spectrum of seston. Second, the particle spectra of pure laboratory cultures of algae are largely unaffected by acid addition. Third, the total volume of calcite given by Coulter measurements is the same as that given by an independent and proven method (particulate inorganic carbon measurements).

Methods

Water collection, pH, alkalinity, and solubility of calcite

Water for all experiments was collected at various depths from an 80-m-deep station (43°05′12″N, 86°24′45″W) located 15 km west of Grand Haven, MI, using large PVC Niskin bottles and emptied into 11-L thermos jugs for transport back to the laboratory. If water was not used for experiments on the day of collection, it was held at lake temperature until analysis the next day. Neither the particle-size spectrum of calcite nor that of other seston was changed significantly by holding the water for 1 d.

pH was measured with a digital pH meter (calibrated at pH 4 and 7) with an automatic temperature compensator. Carbonate alkalinity was determined by potentiometric titrations (Strickland and Parsons 1968), and in situ temperatures were measured with reversing thermometers. Solubility of calcite in Lake Michigan was indicated by the ratio IAP/K_{sp} ((ion activity product of Ca^{2+} and CO_3^{2-})/(solubility product of calcite)), calculated from pH, carbonate alkalinity ($\bar{X} \pm \text{SD} = 107 \pm 4$ $mg \cdot L^{-1}$), and temperature using the equations of Eadie (1980) for the ionic content of Lake Michigan (Torrey 1976). A value greater than 1 indicates supersaturation of $CaCO_3$.

Acid concentration

We added 1 mL of 1% (W/V) acetic acid solution to 100 mL of lake water to dissolve the $CaCO_3$ in experiments in 1978. This concentration of acid lowered the pH of Lake Michigan water to 3.8. At this pH, the only species of inorganic carbon should be free CO_2 (Hutchinson 1957). Since acetic acid is commonly used in Lugol solution at this concentration to preserve algae and other seston in lake water (e.g. Vollenweider 1974), we expected that the particle-size spectrum of most algae and other organic seston would be unaffected by the acetic acid addition. Although we were satisfied with this approach, acetic acid addition was replaced by addition of 1.25 mL of a 0.2 *M* HCl solution to 100 mL of lake water in 1979 and later years in anticipation of doing zooplankton feeding experiments (described below) to compare feeding on total lake seston and on seston without calcite. In these experiments the calcite was to be removed by HCl addition followed later by NaOH addition to bring pH back to normal. At this concentration of HCl, the pH of Lake Michigan water was 3.4.

Coulter counter methods

Particle-size spectra were measured with a model TA-II Coulter counter according to the methods given by Vanderploeg (1981a, 1981b). Generally, a combination of 50- and 200-μm aperture tubes was used to measure the spectrum between 1 and 80 μm equivalent spherical diameter of particles in lake water that was prescreened with a 153-μm screen. Only very large particles like zooplankton and large filamentous or colonial algae were removed by the screen.

Acid exposure experiments

Lake seston — In the acid exposure experiments during 1978 and 1979, 153-μm-screened lake water was poured among a number of 200- or 300-mL dark bottles. Bottles without acid served as controls. Typically, experiments were run in triplicate. Bottles were placed on a rotating wheel (0.2–0.5 revolutions$\cdot$min^{-1}) in an incubator set to ambient lake temperature for various exposure times ranging between 15 min and 26 h. At the end of the exposure, a subsample from each bottle was analyzed with the 200-μm tube; another subsample was taken, screened through a 53-μm screen, and analyzed with the 50-μm tube. Screening with the 53-μm screen was necessary to prevent particles from plugging the 50-μm aperture. Before analysis, enough filtered NaCl solution was added to the analysis beaker to make a 0.5% NaCl solution. After adding salt, samples were stirred for 4 min to allow air bubbles to escape from suspension.

Later we became interested in knowing precisely the minimum exposure time required to dissolve calcite crystals so as to minimize possible effects of acid on certain algae (see Results and Discussion) as well as minimize the time spent doing the analyses. In these experiments, exposure time to acid was limited to minutes or seconds. To do this, untreated screened lake water was placed in a Coulter analysis beaker with graduated volume marks, and replicate Coulter counts were made to determine particle concentration of the total seston. Then the appropriate amount of HCl was added to the same beaker for dissolution of the crystals, and the sample was quickly recounted. Minimum exposure time was also estimated by treating 100-mL samples of offshore Lake Michigan water from the 4-m depth with HCl for various exposure times and filtering the samples through 25-mm-diameter Millipore HAWG filters. After filtration, 30 mL of 0.2-μm- filtered lake water (pH = 8.4) was sucked through the filter to stop the action of the acid. The membrane filters were placed on microscope slides, dried, and cleared with oil for later visual examination (Moore 1963) for calcite crystals. Later, we suspected that action of the HCl might not be stopped by the final lake water rinse; therefore, we also suspended pure calcite in filtered lake water and did Coulter counts after various acid exposure periods to determine how much calcite remained.

To prepare the calcite suspensions, reagent-grade $CaCO_3$ powder was added to 0.22-μm-filtered lake water to make a concentrated calcite slurry, which was filtered through a 53-μm screen to remove large calcite particles. The slurry was placed in a sonic bath for 5 min. The slurry, minus what had settled to the bottom, was poured into another beaker and sonified again for 5 min. A small sample of the slurry was taken to check that the spectrum shape was similar to the average normalized spectrum for Lake Michigan before using it in the experiment. Actual settling times (while being sonified) to obtain the desired spectrum varied. Some of this slurry was then diluted into filtered lake water to produce the final particle-size spectrum.

Laboratory cultures — To verify that HCl addition had no effect on the algal component of the seston, we tested the effect of HCl addition on pure cultures of the diatom *Cyclotella meneghiniana* (CYOH2 from S. S. Kilham), the green alga *Chlorella vulgaris* var. *viridis* (UTEX 396), the green flagellated alga *Chlamydomonas* sp. (UTEX 795), and the blue-green alga *Synechococcus elongatus* (UTEX 563). Subsamples of pure cultures of exponential-phase algae grown in WC medium (Guillard and Lorenzen 1972) without buffer were transferred into 0.2-μm- filtered hypolimnetic water. Volume concentration of the suspensions was approximately the same or slightly higher than the total concentration of seston usually found in Lake Michigan water. The algae remained in the dark for about 16 h. Afterwards, each algal suspension was carefully split between two bottles, a control and an HCl-treated bottle.

Particulate inorganic carbon measurements

As an independent means of verifying the Coulter measurements of the calcite particle-size spectrum with natural seston, volume concentration of calcite was predicted from particulate inorganic carbon (PIC) measurements. Subsamples (usually 100 mL) of the unacidified, screened (153 μm) lake water were filtered through acid-washed, distilled-water-rinsed, precombusted (400°C, 4 h) 25-mm Gelman A/E glass fiber filters. The filters were placed in 10-mL septum bottles that were precleaned the same way as the filters. Precleaned filters (without sample residue) in septum bottles served as blanks. Samples were combusted at 250°C within 24 h of collection and, after cooling, were capped with acid-rinsed septum caps. The samples were run on an Oceanography International carbon analyzer (nondispersive infrared, CO_2 sensitive) after adding 5 mL of 6% H_3PO_4. PIC concentration (grams C per litre) was converted to volume concentration (cubic millimetres per litre) of calcite by assuming the crystals were pure calcite, having a density of 2.715 g$\cdot$cm^{-3}.

Results and Discussion

After dilute acetic acid was added to the early September lake water, particle concentration rapidly decreased initially (see the 20-min exposure spectrum), and then only slightly during the next few hours (Fig. 1A).

The particle-size spectrum of calcite calculated by subtracting the 4-h acid-treated spectrum from the initial (control spectrum) is shown in Fig. 1B. Results of Fig. 1A show that time of acid exposure had little effect on calculated calcite spectrum. Close agreement between total volume of calcite predicted by PIC measurements and the Coulter counter was obtained for acid exposure periods ranging between 20 min and 11 h (Table 1).

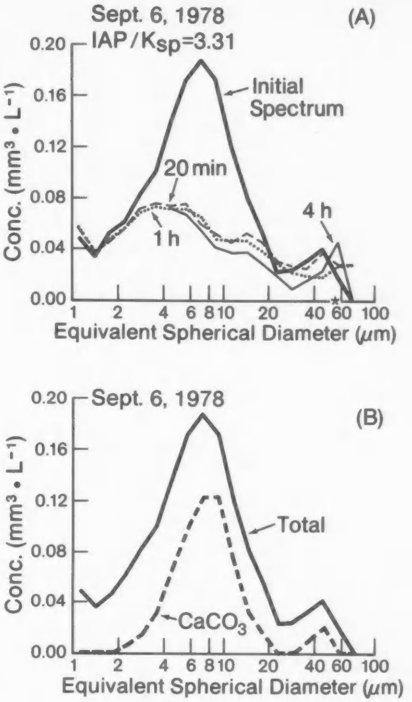

FIG. 1. Particle-size spectrum of epilimnetic water after various exposure times to acetic acid during early September 1978. Date of collection and IAP/K$_{sp}$ ratio of the water are shown on the figure. Depth (*D*), = 4 m, temperature (*T*) = 20°C, and pH = 8.51 at time of collection. The asterisk on the abscissa indicates that for particles of this size and larger the percent error on particle concentration is greater than 25% (Vanderploeg 1981a). (A) Effect of 20 min, 1-h, and 4-h exposures; (B) total (initial) particle-size spectrum and particle-size spectrum of calcite calculated by subtracting the 4-h acid-treated spectrum from the initial spectrum.

Experience gained from using the Coulter counter to measure seston concentrations in grazing experiments (Vanderploeg 1981a) at different times of the year showed that particle concentration decreased by roughly 10% in some parts of the particle-size spectrum during a 24-h period in 300-mL bottles. This result, and the rapid initial decrease of particle concentration after acid addition, suggested that acid exposure should generally be limited to less than about 4 h for accurate results and that controls should preferably be analyzed at the same time as acid-treated bottles. This procedure was provisionally adopted for all but a few special experiments starting in October 1978. An example of particle sticking can be seen in the HCl addition experiment of July 19, 1979 (Fig. 2). Note that the concentration of particles in the 6-h control is slightly lower than that in the initial bottles, the control bottles which were analyzed at the beginning of the experiment. Parallel to the decrease in concentration with time in the controls, the concentration for the 10-h treatment with acid is slightly lower than that in the 6-h treatment.

The 4-h HCl addition method, with the controls analyzed at the end of the experiment with acid-treated experimentals, was tested during August and September 1979 (Fig. 3 and 4; Table 1). Excellent agreement between calcite predicted by the Coulter counter with that by the PIC method was obtained (Table 1).

One of the motives for measuring particle-size spectra during seasons when calcite concentration was low was to determine if acid had any effect on the particle-size spectrum of algae and other organic seston. Late spring is an ideal time for this experiment in Lake Michigan. First, stratification of the water column has started so that resuspended sediments do not introduce carbonates into surface waters. Second, water temperature and pH are low enough to prevent autogenic precipitation of calcite, since IAP/K$_{sp}$ < 1 (Fig. 5A). Late fall is another good time, although one would expect some calcite contribution either from resuspension or from autogenic precipitation in earlier months. The acid addition experiments done at these times, June 1979 (Fig. 5A) and November 1978 (Fig. 5B), demonstrate that acid addition, either HCl or acetic acid, had little effect on the particle-size spectrum of algae and other organic lake seston. These results were corroborated by experiments with different commonly cultured species of algae (Fig. 6). However, in both the experiments with seston and algae, some of the spectra were shifted slightly to the right (Fig. 5 and 6). This probably represents a slight tendency for the cells to clump at low pH. Effects of this magnitude are negligible for estimating the calcite spectrum when it is a significant contributor to the total spectrum.

In addition to the usual acid addition and PIC experiments (Table 1), we examined the effect of very short exposures of HCl to the particle-size spectrum during mid-September 1980 (Fig. 7) to get an accurate estimate of minimum exposure time. The ratio of calcite seen by the Coulter counts relative to the carbon analyzer was 1.34 and 1.30, respectively, for the 0.9- to 3-h duration experiments with epilimnetic water of September 15 and 18, 1980. These values are appreciably greater and in the latter case statistically greater ($P < 0.05$) than the expected value of 1. Better agreement between methods was seen for September 15, 1980, on hypolimnetic water exposed to acid for a considerably shorter time: the Coulter counter to carbon analyzer ratio was 1.06, a value not significantly greater than 1. Figure 7 shows that the acid-labile component starts to disappear very quickly, but that there is considerable difference between 15- to 18-min and 1- to 3-h spectra. Gross microscopic examination showed that the algal composition of the epilimnetic and hypolimnetic water was very similar. Algal composition was different in September 1980 from other years in that colonial blue-green algae (*Gomphosphaeria lacustris* var. *compacta* and *Coelosphaerium küetzegianum*) and the colonial crysophyte *Uroglenopsis* were dominants. We previously observed that the mucilage in these colonial forms slowly dissolves when exposed to acidic Lugol solution. Thus, the disagreement between Coulter and PIC results may have been caused by slowly dissolving algal components.

Examination of calcite remaining on the oil-cleared membrane filters after acidification of the lake seston suggested that calcite was dissolved by the HCl in about 1.5 min. Thus, we adopted the technique of doing Coulter counts on a beaker of lake water, then adding 1.25 mL of 0.2 *M* HCl per 100 mL of lake water remaining in a beaker, and then repeating the Coulter analyses after 1.5 min as detailed under Methods. Because of counting time for triplicate analyses, the acid exposure period was usually 1.5–6 min.

To test the technique, we used it and the long acid exposure method to determine calcite concentration in epilimnetic water of September 30, 1980 (Table 1). Algal composition had not

TABLE 1. Comparison of volume of calcite given by the Coulter counter and by the carbon analyzer. Control bottles were analyzed at time experiment was set up (B) or and the end (E). *Significant difference ($P < 0.05$, t-test) between Coulter and carbon analyzer results.

Date	Sample[a]	Acid	Exposure time to acid	Time controls analyzed	Total calcite concentration ($mm^3 \cdot L^{-1}$) Coulter counter	Carbon analyzer	Coulter counter/carbon analyzer
Sept. 6, 1978	Epilimnion, 1-d-old	AcCOOH	20–45 min	B	0.54±0.02(3)	0.48±0.01(3)	1.13
Sept. 6, 1978	Epilimnion, 1-d-old	AcCOOH	1.0–1.5 h	B	0.54±0.02(3)	0.48±0.01(3)	1.13
Sept. 7, 1978	Epilimnion, 2-d-old	AcCOOH	4.5–5.5 h	B	0.53±0.01(4)	0.48±0.01(3)	1.10
Sept. 7, 1978	Epilimnion, 2-d-old	AcCOOH	11.5–12.0 h	B	0.53±0.01(4)	0.48±0.01(3)	1.10
Aug. 10, 1979	Epilimnion	HCl	5.0–6.0 h	E	0.06±0.03(3)	0.10±0.03(6)	0.60
Aug. 22, 1979	Epilimnion	HCl	4.7–6.7 h	E	0.09±0.05(2)	0.10±0.03(3)	0.90
Sept. 11, 1979	Epilimnion	HCl	4.8–6.8 h	E	0.25±0.03(3)	0.19±0.03(4)	1.32
Sept. 11, 1979	Metalimnion (18 m)	HCl	4.8–6.8 h	E	0.23±0.02(3)	0.19±0.03(4)	1.21
Sept. 12, 1979	Epilimnion, 1-d-old	HCl	4.0–6.0 h	E	0.25±0.02(3)	0.22±0.03(3)	1.14
Sept. 15, 1980	Epilimnion	HCl	0.9–2.9 h	E	0.51±0.02(5)	0.38±0.03(3)	1.34
Sept. 15, 1980	Hypolimnion (35 m)	HCl	15 min – 2.1 h	E	0.38±0.02(6)	0.36±0.02(3)	1.06
Sept. 18, 1980	Epilimnion, 3-d-old	HCl	1.5–3.0 h	E	0.30±0.01(3)	0.23±0.002(3)	1.30*
Sept. 30, 1980	Epilimnion, 1-d-old	HCl	1.5–6.0 min	B	0.29±0.01(6)	0.27±0.004(4)	1.07
Sept. 30, 1980	Epilimnion, 1-d-old	HCl	1.0–2.0 h	E	0.35±0.01(6)	0.27±0.004(4)	1.30*
Sept. 30, 1980	Hypolimnion (40 m), 1-d-old	HCl	1.5–6.0 min	B	0.11±0.02(3)	0.12±0.002(3)	0.92
Aug. 25, 1981	Epilimnion	HCl	1.5–6.0 min	B	0.31±0.02(4)	0.44±0.01(3)	0.70*
Sept. 6, 1981	Epilimnion	HCl	1.5–6.0 min	B	0.39±0.03(6)	0.69±0.03(4)	0.56*
Sept. 3, 1982	Epilimnion, 1-d-old	HCl	15–25 min	E	0.32±0.02(4)	0.37±0.004(4)	0.86
Sept. 7, 1982	Epilimnion	HCl	15–25 min	E	0.26±0.04(4)	0.28±0.005(4)	0.93
Sept. 14, 1983	Epilimnion	HCl	15–28 min	E	0.087±0.005(2)	0.103±0.007(4)	0.84

[a] All samples from the epilimnion were taken from 4 m.

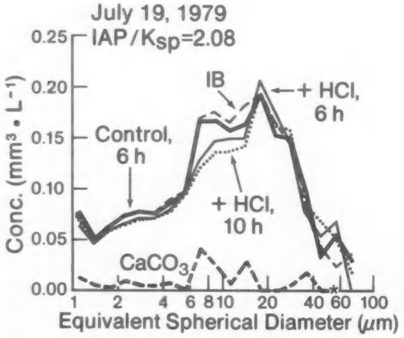

FIG. 2. Effect of various exposure times of HCl on epilimnetic water during July 1979 and effect of time on changes in control (untreated) bottles ($D = 4$ m, $T = 12$°C, pH = 8.52). "Initial bottle (IB)" is control bottles analyzed at the start of the experiment. "Control, 6 h" is control bottles analyzed 6 h after the start of the experiment. The $CaCO_3$ spectrum is the difference between 6-h control and 6-h acid-treated spectra.

changed much from the experiments done earlier in the month. Excellent agreement between PIC and Coulter count results was obtained with the short exposure method, and again the longer exposure method resulted in a somewhat higher prediction for the Coulter counter relative to the carbon analyzer (Table 1). The short exposure method also gave good results for hypolimnetic water on this date (Table 1).

Calcite concentration was again measured by the short acid exposure and PIC methods during early September 1981 (Table 1; Fig. 8). This time the Coulter technique significantly underestimated the calcite concentration, implying that the acid

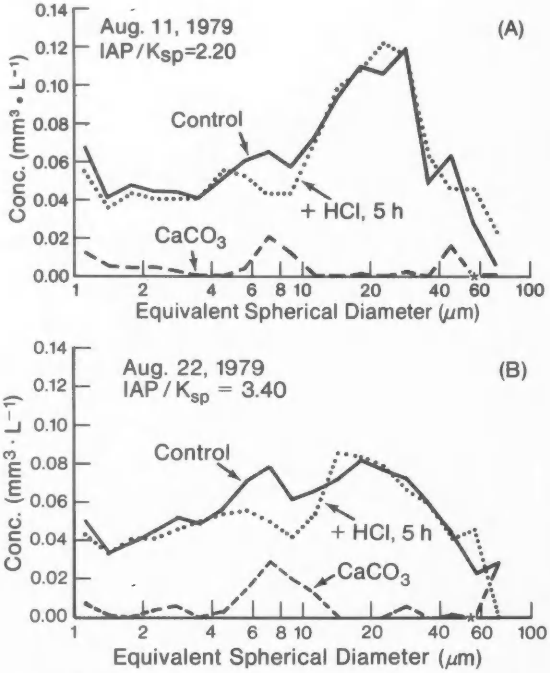

FIG. 3. Contribution of calcite to the total (control) particle-size spectrum of epilimnetic water during August 1979 calculated by the difference between control and acid-treated spectra. (A) Early August ($D = 4$ m, $T = 19$°C, pH = 8.33); (B) late August ($D = 4$ m, $T = 17$°C, pH = 8.57).

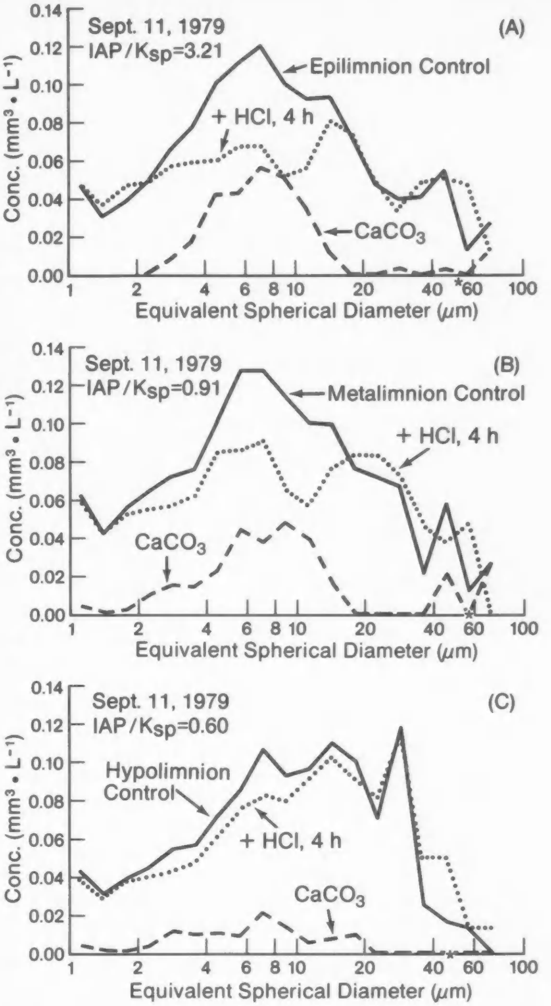

FIG. 4. Contribution of calcite to the total particle-size spectrum in September for the (A) epilimnion (D = 4 m, T = 17°C, pH = 8.54), (B) metalimnion (D = 18 m, T = 8°C, pH = 8.21), and (C) hypolimnion (D = 30 m, T = 6°C, pH = 8.07).

exposure period was too short. The results of the experiment with hypolimnetic water of September 15, 1980, suggested that an exposure period longer than 15 min would be acceptable. This was verified by experiments with pure suspensions of calcite in lake water which showed that 15 min is necessary to dissolve large (>10μm) calcite particles. From these results, we finally settled on an exposure time of 15–30 min. The 15- to 30-min exposure time was tried in September 1982 (Fig.9). Coulter and PIC results were quite close (Table 1). The acid addition method was repeated in September 1983. Although calcite concentration was very low, good agreement between Coulter and PIC results was obtained (Table 1). We therefore recommend that acid exposures of 15–30 min be used, since this period is long enough to dissolve the calcite yet short enough to prevent dissolution of the mucilage sheath around acid-sensitive algae.

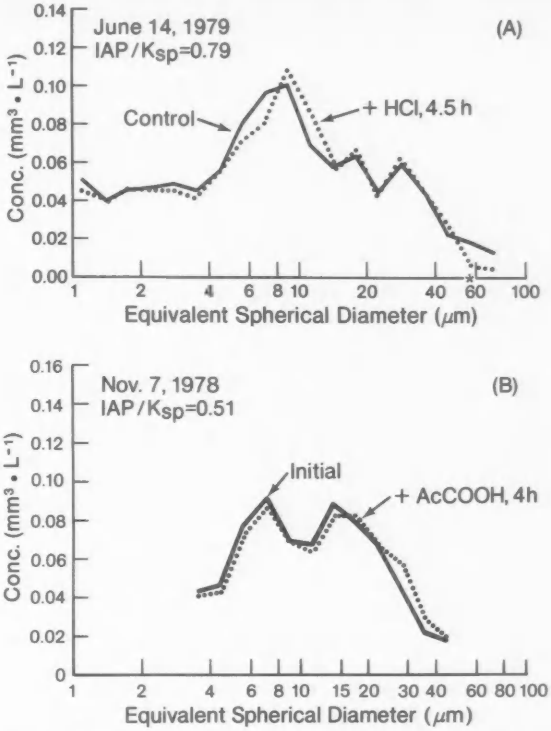

FIG. 5. Effect of acid addition during spring after stratification and during fall overturn. Differences between control and acid-treated spectra were too small to plot meaningful calcite spectra. (A) Spring (D = 4 m, T = 7°C, pH = 8.17); (B) fall overturn (D = 4 m, T = 11°C, pH = 7.89).

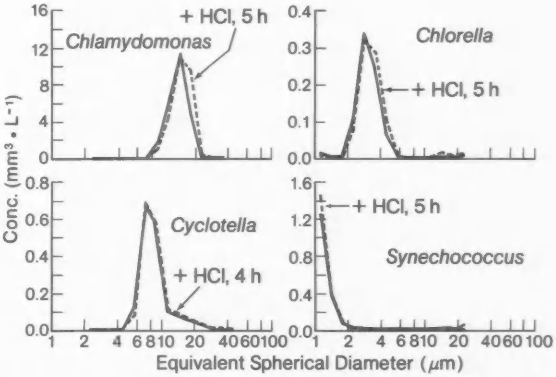

FIG. 6. Effect of HCl addition on particle-size spectra of different kinds of algae.

Contribution of Calcite to the Particle-Size Spectrum

The calcite spectra obtained by the acid addition technique illustrate the seasonal development and decline of the whiting phenomenon (Fig. 1–5 and 7–9). The calcite spectrum was only a modest part of the total particle-size spectrum of the epilimnion during July (Fig. 2), early August (Fig. 3A), and late

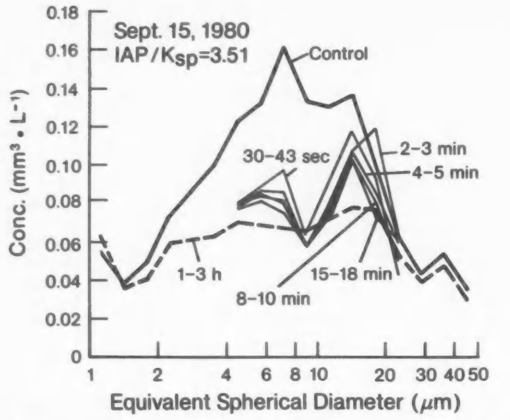

FIG. 7. Effect on long-term (1–3 h) and short-term exposure to HCl on epilimnetic water during mid-September 1980 (D = 4 m, T = 20°C, pH = 8.53). Results for water receiving short-term acid treatment are shown only for particles >4μm because only the 200-μm aperture was used.

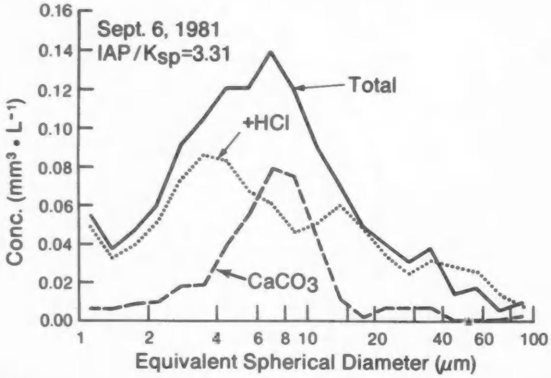

FIG. 8. Effect of short-term exposure to HCl and the calculated calcite spectrum for epilimnetic water during early September 1981 (D = 4 m, T = 21°C, pH = 8.46).

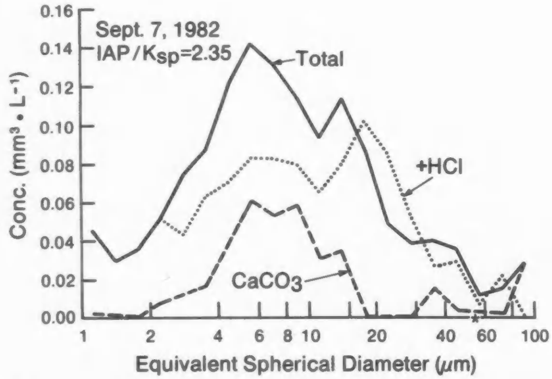

FIG. 9. Contribution of calcite to the particle-size spectrum of epilimnetic seston during early September 1982 (D = 4m, T = 18°C, pH = 8.36).

August (Fig. 3B), when respective IAP/K_{sp} ratios were 2.1, 2.2, and 3.4 (Fig. 2 and 3). By early September (Fig. 4), when concentration was maximum, the IAP/K_{sp} ratio was 3.2; thus, there was a lag of 2 mo after the IAP/K_{sp} ratio was greater than 2 before calcite was a major contributor to the particle-size spectrum. A similar lag is suggested from extensive data obtained by satellite imagery (Strong and Eadie 1978) and by PIC measurement (J. Eadie, L. Goad, and A. E. Strong, in prep.). The decrease in the calcite spectrum with depth for September (Fig. 4) was a result of the corrosive (IAP/$K_{sp} < 1$) properties of deep water associated with its lower pH and temperature. During fall overturn (Fig. 5B), conditions are corrosive (IAP/K_{sp} = 0.51, Fig. 5B) for calcite at 4m; thus, little remains in the water. Probably calcite concentration dropped very quickly at overturn with the admixture of cool, low-pH water from the hypolimnion that contained little calcite to begin with, even during September (Fig. 4C).

Our data and observations of others based on Secchi disc transparency (Ladewski and Stoermer 1973; Rockwell et al 1980), satellite imagery (Strong and Eadie 1978), and PIC measurements (J. Eadie, L. Goad, and A. E. Strong, in prep.) showed that maximum or near-maximum intensity of whitings occurs during early September and usually remains high until late September. Thus, we determined the contribution of calcite to the total particle-size spectrum during September for 1978–83 and 1985 to get an estimate of the maximum contribution of calcite and its year-to-year variability (Table 2). The contribution of calcite to the total particle-size spectrum in the epilimnion during September varied greatly, with values ranging between 12 and 56%. If we exclude the anomolous value of 12% for 1983, when the whitings were not even visible to observers shipboard (D. Scavia and G. L. Fahnenstiel, GLERL/NOAA, pers. comm.), the contribution varied between 20 and 56%, values equivalent to calcite levels of 0.26 and 0.69 $mm^3 \cdot L^{-1}$ (Table 2).

The key variable in determining year-to-year variation in the strength of the whiting phenomenon is summer primary production. IAP/K_{sp} for calcite in Lake Michigan (J. Eadie, L. Goad, and A. E. Strong, in prep.) is very sensitive to pH and is less sensitive to temperature. This is the same pattern that is well known for calcite equilibria in distilled water and in other hardwater lakes (e.g. Hutchinson 1957; Kelts and Hsu 1978). Since elevated pH is caused by CO_2 uptake by phytoplankton during photosynthesis (e.g. Verduin 1956; Kelts and Hsu 1978; Scavia 1980), it follows that primary production is the key variable. Epilimnetic concentration of chlorophyll was unusually low during summer 1983 (Scavia et al. 1986; D. Scavia, GLERL/NOAA, unpubl. data). Low CO_2 uptake would be expected with this low standing stock. Corroborating this are the measurements of Fahnenstiel and Scavia (1987, tables 1 and 2) showing lower primary production in 1983 than in 1984.

The reduced standing stock of phytoplankton in 1983 was attributed to a causal chain resulting from the stocking of salmon (Scavia et al. 1986). Increasing populations of stocked salmonids reduced populations of the planktivorous alewife (*Alosa pseudoharangus*), which allowed large *Daphnia* to flourish. *Daphnia* spp., more voracious and nonselective grazers than the formerly dominant *Diaptomus* spp., reduced the concentration of algae. It seems probable that this same causal chain was responsible for lack of an appreciable calcite whiting in 1983.

An interesting finding of our study is that although epilimnetic calcite concentration during September varied considerably from year to year, the particle-size spectra in the epilimnion of

TABLE 2. Contribution of calcite to the total particle-size spectrum during September. The ratio IAP/K_{sp} is (ion activity product of Ca^{2+} and CO_3^{2-})/(solubility product of calcite). Values of the ratio greater than 1 indicate supersaturation of $CaCO_3$.

					Concentration by volume ($mm^3 \cdot L^{-1}$)		
Date	Depth	Temperature (°C)	pH	IAP/K_{sp}	Total seston	Calcite	Percent calcite
Sept. 6, 1978	Epilimnion (4 m)	20	8.51	3.19	1.42	0.54	38
Sept. 19, 1978	Epilimnion (4 m)	20	8.52	3.50	1.23	0.54	44
Sept. 11, 1979	Epilimnion (4 m)	17	8.54	3.21	1.19	0.25	21
Sept. 11, 1979	Metalimnion (18 m)	8	8.21	0.91	1.34	0.23	17
Sept. 11, 1979	Hypolimnion (25 m)	6	8.07	0.60	1.17	0.04	3
Sept. 15, 1980	Epilimnion (4 m)	20	8.53	3.51	1.56	0.38	24
Sept. 15, 1980	Hypolimnion (35 m)	14	8.39	1.97	1.49	0.36	24
Sept. 30, 1980	Epilimnion (4 m)	16	8.42	2.23	1.26	0.29	23
Sept. 30, 1980	Hypolimnion (40 m)	13	8.05	0.87	0.80	0.11	14
Sept. 6, 1981	Epilimnion (4 m)	21	8.46	3.31	1.23	0.69	56
Sept. 7, 1982	Epilimnion (4 m)	18	8.36	2.35	1.27	0.26	20
Sept. 15, 1983	Epilimnion (4 m)	21	—	—	0.75	0.087	12
Sept. 23, 1985	Epilimnion (4 m)	18	—	—	1.41	0.66	47
Mean Sept. value for epilimnion during whiting years (1978–82 and 1985)		19	8.47	3.04	1.31	0.46	35

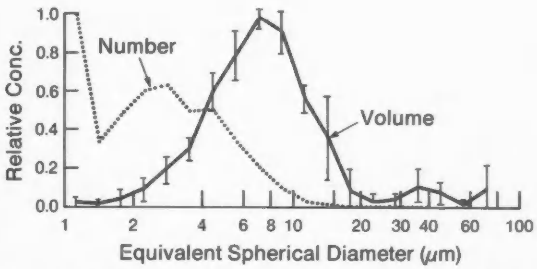

FIG. 10. Relative (normalized) concentrations of particle number and volume (±SD) in the different size categories during September 1978–82 and 1985.

September during whitings (1978-82) were very similar (Fig. 1, 4, 8, and 9). This similarity is also evident from the modest standard deviations on the average normalized volume spectrum of these same results (Fig. 10). Little is known about particle-size spectra of $CaCO_3$ in other lakes. The weight-averaged equivalent particle diameter reported by Brunskill (1969) for Fayetteville Green Lake can be compared with the location of the mode in our particle volume spectrum (Fig. 10). Brunskill's mean particle diameter of 6 μm, determined from microscopic counting of 800 particles, compares well with our modal diameter of 7 μm (Fig. 10). Koschel et al. (1983) reported crystals ranging in size between 0.6 and 5 μm. Weidemann et al. (1985) reported approximately equal number of particles among the size-classes 4-8, 8-16, and >16 μm in Otisco Lake, a result very different from ours (number curve of Fig. 10); also, their crystals had a curious dendritic pattern of arrangement. Note that the Coulter counter could be used to get an accurate estimate of the volumes and equivalent spherical diameter of these dendritic crystals; however, the equivalent spherical diameter would be smaller than greatest axial linear dimension of the crystals because of their shape. It would be of great interest to use the Coulter method to determine the size spectrum of calcite in other lakes to see what generalizations emerge.

The acid-treated spectrum is largely composed of phytoplankton and plankton-derived detritus and, as such, is the major food of the herbivorous and omnivorous zooplankton. If during September we considered the total (unacidified) concentration of particles as food for zooplankton, we could greatly overestimate the food available to zooplankton because calcite can be a large percentage of the total seston (Table 2). Moreover, we would have a biased perception of particle-size spectrum shape. The shape of the total (unacidified) spectrum for the epilimnion during September usually shows a sharp peak at 7 μm (Fig. 1, 4, 7, 8, and 9). Such a sharp peak has been associated with changing seasons in temperate lakes (Sprules et al. 1983) and in coastal areas and higher latitudes of the ocean (Sheldon et al. 1972, 1977; Sprules et al. 1983). In contrast, many of the "food" spectra, or acid-treated spectra, for these same experiments are relatively flat, a condition associated with an unchanging habitat such as the lower latitude oceans (Sheldon et al. 1972, 1977; Sprules et al. 1983). September is near the end of the hydrodynamically stable period in Lake Michigan that started with onset of stratification during June. Zooplankton grazing during September is at or near maximum, and algal population size is thought to be regulated by zooplankton grazing and nutrient recycling from grazing (e.g. Scavia 1979; Scavia and Fahnenstiel 1987). Intuitively, flat spectra would be expected, since zooplankton grazing and particle production observed in feeding experiments with natural seston tends to level peaks in the spectra (e.g. Vanderploeg 1981a). Thus, the flat acid-treated spectrum, rather than the peaked untreated spectrum, conforms to our expectations for the shape of the spectrum during late summer.

Calcite–Plankton Interactions

We have seen how food web dynamics affect the calcite system. Now we explore potential effects of calcite on plankton dynamics. A major motivation for examining the calcite–zooplankton interactions was finding large quantities of calcite in fecal pellets (Fig. 11). First, this suggested that significant

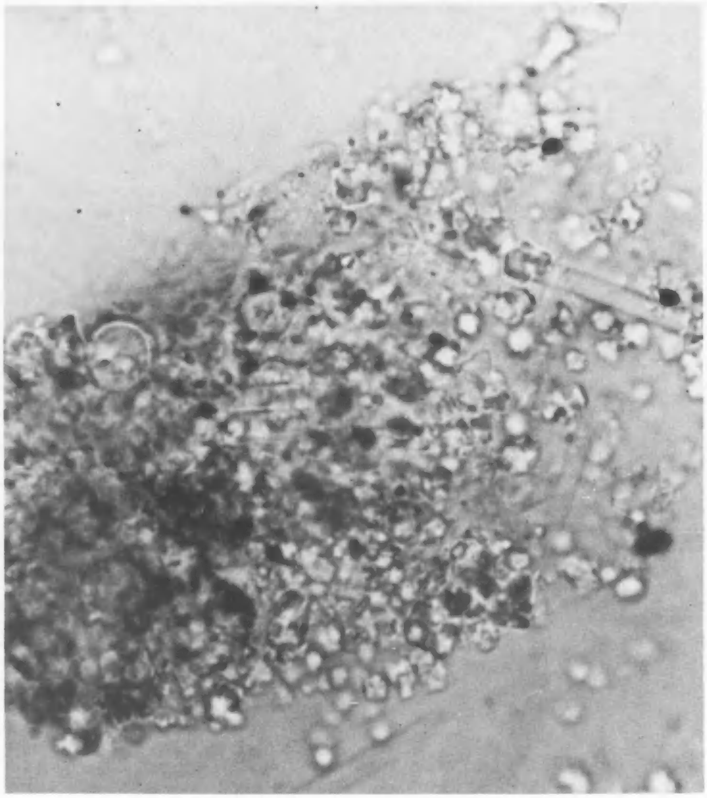

FIG. 11. A broken fecal pellet showing its contents: calcite crystals and diatom frustules. Width of the pellet is 30 μm.

quantities of calcite were ingested by suspension-feeding copepods. We were concerned whether zooplankton feeding might be adversely affected by the presence of the calcite. We expected suspension-feeding copepods, which capture particles actively in response to their scent as well as passively by filtration (Vanderploeg and Paffenhöfer 1985), to ingest less calcite per unit of food intake than the filter-feeding cladocerans. This could have implications to seasonal succession of zooplankton if copepods were less affected by the calcite than cladocerans. Therefore, we did experiments to determine effects of calcite on feeding of a cladoceran, *Daphnia pulex*, and a copepod, *Diaptomus sicilis*. We also wondered whether ingestion with subsequent gut passage dissolved the calcite. This could be a mechanism of calcite destruction.

Second, calcite in fecal pellets suggested that fecal pellet sinking rate would increase because of increased specific gravity of the pellets. This could not only affect removal rate of nutrients sorbed to the calcite, but also N, P, and Si associated with algal remains in fecal pellets (e.g. Ferrante and Parker 1978). Therefore we estimated the effect of calcite inclusion on fecal pellet sinking rate.

Another potentially important means for calcite to affect nutrient dynamics is through coprecipitation or sorbtion and subsequent sinking of the crystals. From estimates of downward flux of calcite from calcite concentration and Stokes settling velocity and from information on phosphate coprecipitation from laboratory experiments (Ishikawa and Ichikuni 1981), we estimated the removal rate coefficient of phosphate, the limiting nutrient in Lake Michigan.

Effects of light scattering on extinction of light can be predicted for a mineral suspensoid if its concentration and particle-size spectrum are known (e.g. Kirk 1985). We examined the light scattering properties of calcite, and we demonstrate why in Lake Michigan it has only a modest effect on decreasing light penetration, but has a large effect on decreasing Secchi depth.

Methods

Calcite–zooplankton feeding interactions

Laboratory experiments with Daphnia pulex – Both the effect of calcite on feeding rate of *D. pulex* and the effect of *D. pulex* feeding on calcite were explored using the laboratory-cultured diatom *Cyclotella meneghiniana* (Culture CYOH2 of S. S. Kilham) and laboratory-prepared suspensions of calcite having volume spectra similar to the mean normalized volume spectrum of calcite in Lake Michigan (Fig. 10). We examined the effect of *D. pulex* feeding on calcite, since calcite by its ingestion and subsequent gut passage might be dissolved. In these experiments, various concentrations of calcite were added to a fixed, 0.8 $mm^3 \cdot L^{-1}$ concentration of *C. meneghiniana*. This concentration of algae falls within the low to middle range

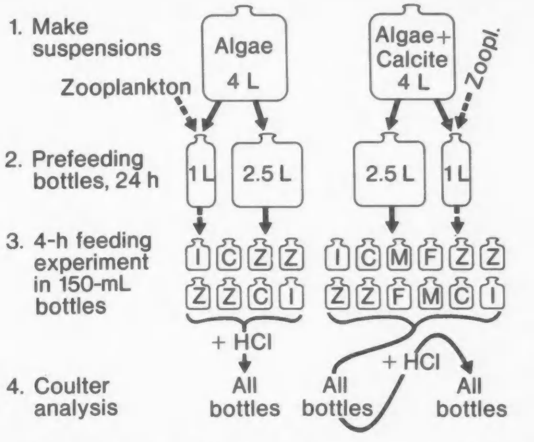

FIG. 12. Design of paired feeding experiments to evaluate the effect of calcite on feeding rate of *Daphnia pulex* on algae and the effect of *D. pulex* feeding on calcite concentration. At step 1, 4-L suspensions of *Cyclotella* and *Cyclotella* plus calcite were prepared. During step 2, the prefeeding period, *Daphnia pulex* fed in appropriate prefeeding suspensions. Ungrazed water and prefed animals from step 2 were put together in step 3, the feeding experiment. In the *Cyclotella*-only treatment the usual initial (I), control (C), and experimental bottles with zooplankton (Z) were used. In the *Cyclotella*-plus-calcite treatment, two different kinds of control bottles with zooplankton were added: the M bottles and F bottles, from which subsamples of water were quickly drawn from the middle of the bottle using a pipette at the midpoint (M bottles) and end of the experiment (F bottles). These bottles allowed monitoring of calcite available in suspension for the zooplankton. Calcite available to the zooplankton during the feeding experiment was taken as that measured in the M bottles.

of total food concentrations found in Lake Michigan (Vanderploeg et al. 1984).

The experiment used a paired design in which one group of *D. pulex* fed only on algae and the other fed on algae plus calcite (Fig. 12). This design would allow discernment of modest effects of calcite on feeding rate that might be hidden in experiment-to-experiment variation associated with using different groups of animals on different dates (Vanderploeg et al. 1986). The Coulter counter was used to measure volume of particles consumed per unit dry weight of zooplankton using methods detailed by Vanderploeg et al. (1986). Culture of algae and *D. pulex* followed methods given by Vanderploeg et al. (1986) to ensure that *D. pulex* were well fed and in excellent physiological condition before the experiments were started. Experiments were run at 20°C, and algae and calcite were suspended in 0.2-μm-filtered Lake Michigan water. Calcite suspensions, having the particle-size spectrum shape of the average normalized spectrum, were prepared by sonification of pure calcite suspensions as described above for the acid exposure experiments.

The experiments followed traditional experimental design of a prefeeding period of 24 h (Vanderploeg et al. 1984, 1986) followed by a shorter feeding period in which preconditioned *D. pulex* were added to smaller bottles to determine of feeding rate (Fig. 12). The algae-only treatment had the usual duplicate initial and control bottles and four experimental bottles, each containing six *D. pulex* (2.4–3.0 mm total length exclusive of tail spine). The prefeeding period may be especially critical to proper experimental design to allow zooplankton experience in handling the calcite and adjust to its presence. The tendency of the calcite to settle out (Stokes settling velocity $\simeq 16\ \mathrm{cm \cdot h^{-1}}$) in the small feeding bottles necessitated addition of extra control bottles to monitor calcite available to the zooplankton during the feeding period (Fig. 12).

At the appropriate times, subsamples of all bottles from the algae-plus-calcite treatment were analyzed on the Coulter counter to give feeding rate on the total assemblage of particles. Then all bottles received standard acid treatment (1.25 mL of 0.2*M* HCl per 100 mL of sample) and were reanalyzed 30 min later to give feeding rate on the algae only. By this technique, we were able to do a mass balance on the algae and calcite separately. Acid treatment preceded Coulter analysis of the algae-only treatment to ensure a balanced experimental design: calculation of feeding rates on algae from both treatments would be for acid-exposed algae. *Cyclotella meneghiniana* is an ideal alga for these experiments because acid addition does not affect its particle-size spectrum (Fig. 6).

Lake seston experiment with Diaptomus sicilis – The effect of calcite feeding of adult female *D. sicilis*, a dominant Lake Michigan copepod, during a calcite whiting was determined using an introduced radiolabeled alga. A paired experimental design permitted determination of grazing in the total seston and in seston without calcite. Seston without calcite was produced by standard HCl addition for 15 min followed by addition of NaOH to restore pH to normal. Addition of NaOH restored pH levels to normal, so that zooplankton feeding would be observed at normal, ambient pH, but did not cause reprecipitation of calcite, since reprecipitation requires several days at ambient pH.

Cyclotella sp. (UTEX 1269) was chosen as the alga to be labeled because its 8-μm equivalent spherical diameter would be about the same as the mode in the particle-size spectrum of calcite (Fig. 10). Second, we thought that the effect of calcite on ingestion of *Cyclotella* sp. would be intermediate between the extremes anticipated for a large alga captured entirely actively and a small one captured entirely passively (by filtering), since an 8-μm-diameter cell would be captured both actively and passively (Vanderploeg and Paffenhöfer 1985). (*Cyclotella meneghiniana*, which was used in our method evaluation experiments, would have been a suitable alga for these experiments, but we were having trouble culturing it at the time.) It seemed likely that an actively captured large alga, which is ingested immediately after capture, would be relatively unaffected by the calcite, which is probably captured passively because it is inert and would not have a phycosphere of odor (Strickler 1984). A small passively captured alga, collected in a group with other small algae and calcite before ingestion (Paffenhöfer et al. 1982; Vanderploeg and Paffenhöfer 1985), would possibly be rejected along with all members of the group if the group did not have the proper taste.

Diaptomus sicilis were captured at 1200 EDT on September 23, 1985, from net tows from 70 to 30 m, placed in 11-L insulated jugs of upper hypolimnetic (30 m) water, and transported to the laboratory along with a jug of epilimnetic (4 m) water. The water with zooplankton (initial temperature = 10°C) was allowed to warm up slowly during the day to 18°C by 2100 EDT. To precondition the *D. sicilis* to feeding in epilimnetic water, in which we expected to find the higher calcite concentration, we transferred 250 of them into a 2.5-L bottle containing 153-μm-screened epilimnetic water and placed the bottle on a wheel rotating at 1 revolution$\cdot$min^{-1} overnight in an inculator set to 20°C and dim light having a 14 h : 10 h light–

dark cycle. Later Coulter analyses of the lake water showed nearly identical concentrations of calcite in the epilimnion and upper hypolimnion.

The next morning, 5 L of epilimnetic water, which was also kept in the incubator with the preconditioning zooplankton, was screened with a 153-μm screen. From this a 2.5-L bottle of untreated water served as the total seston treatment, and a 2.5-L bottle treated with standard HCl and NaOH addition served as the seston-minus-calcite treatment. Water from each treatment bottle was poured among six 320-mL bottles for the grazing study using the labeled *Cyclotella* sp. Four of the bottles receiving 30 *D. sicilis* served as experimental bottles, and two without served as controls for Coulter counting to determine seston concentration available to the zooplankton. All bottles were placed on the wheel rotating at 1 revolution$\cdot$min^{-1} at the same incubator settings at 1300 EDT. The bottles were always kept in rotation to prevent calcite from settling out. Bottles from both treatments received identical handling. Zooplankton were allowed to feed in the bottles for 2 h before the labeled algae were added.

Usual experimental protocol for labeling the algae, conducting the experiment,and analyzing the results using liquid scintillation counting of the ^{14}C label was used (e.g. Peters 1984). Two days before the experiment, 200 mL of exponential-phase *Cyclotella* sp. was labeled by placing in it a sterile 500-mL bottle along with 3.7 MBq of [^{14}C]NaHCO$_3$ under a light climate of 70 μEin$\cdot$m$^{-2}\cdot$s^{-1} at 20°C on a 16 h : 8 H light–dark cycle. On the day of the experiment, the labeled *Cyclotella* was centrifuged and washed three times in filtered lake water to remove labeled medium. Coulter analyses of the washed, labeled cell suspension verified that the size spectrum of algae was unaffected by the centrifugation and washing and indicated how much labeled cell suspension should be added to the experimental bottles. Approximately 1 mL of labeled *Cyclotella* suspension was added to each of the experimental bottles; this addition increased the particle concentration by 0.05 mm$^3\cdot$L^{-1}, a value less than 10% of the total noncalcarious seston. After the labeled algae were introduced, feeding was allowed to proceed for 7 min, at which time the bottle was removed from the wheel and its contents poured through a 200-μm screen to collect the zooplankton. The zooplankton were narcotized in club soda and immediately transferred to liquid scintillation vials containing 1 mL of Protosol to dissolve the zooplankton and their ingested labeled algae. Subsamples (2-mL) of water that passed through the screen were filtered on 22-mm-diameter, 0.45-μm-pore size Millipore filters and placed in liquid scintillation vials for determination of ^{14}C concentration in the algae. Aquasol was used as the liquid scintillation cocktail for both the algae and zooplankton. Zooplankton were allowed to dissolve for 2 d at 45°C in the Protosol before liquid scintillation cocktail was added. The liquid scintillation vials were kept for 2 d in the dark before counting so that interfering chemoluminescence would disappear.

The paired experimental design for evaluating effects of calcite on feeding of *D. sicilis* on natural seston implicitly assumes the acid and base addition to the seston will not affect its palatability as food for *Diaptomus*. This assumption was verified by comparing grazing rate on labeled *Cyclotella* sp. introduced into untreated and into acid-plus-base treated suspensions of algae. A mixture of 0.2 mm$^3\cdot$L^{-1} each of *Chamydomonas oblonga* (UTEX 219), *C. proteus (UTEX 26),* and *Chlamydomonas* sp. (UTEX 796) in 0.2-μm-filtered lake water was used to simulate a particle-size spectrum of natural algae; respective equivalent spherical diameters of the algae are 4, 6, and 12 μm. Results of this experiment are given below along with results from the seston experiment.

Calculation of light scattering by calcite

The extinction coefficient, *b*, per unit length for light scattering of nonabsorbing particles of radius *r* is given by

$$b = N\pi r^2 Q_{\text{scat}}$$

where N = number of particles per unit volume and Q_{scat} = scattering cross-section of the particle divided by its cross-sectional area (πr^2 for a spherical particle of radius r). Anomolous diffraction theory of van de Hulst (1957) was used (Kirk 1983, equation 3) to predict Q_{scat} of calcite. These relations were applied to each Coulter particle-size category (channel) to give *b* for each channel and then summed over all channels. For purposes of simplification, *b* was calculated for green light (550 nm), which is typically one of the most penetrating wavelengths in natural waters (Kirk 1983, 1985); also, *b* is not greatly sensitive to wavelength.

Calcite and nutrient dynamics

Sinking rate and downward flux of calcite — Stokes settling velocities (e.g. Sverdrup et al. 1942, p. 956) were determined for calcite in each particle-size category of the normalized calcite spectrum (Fig. 10) for 20°C, the temperature of the epilimnion in September. A weight-averaged sinking rate for the total spectrum was determined from sinking rates and relative concentrations of calcite in each of the channels. From sinking rate, the depth of the epilimnion (20 m), and calcite concentration, the fraction of calcite lost per day from the epilimnion was estimated. Flux (grams per square metre per day) was estimated from the product of fraction lost (per day) times the amount of calcite in the epilimnion (grams per square metre).

Phosphate coprecipitation — Phosphate coprecipitation with calcite was estimated from the laboratory experiments of Ishikawa and Ichikuni (1981) that were performed to identify the mechanism of phosphate coprecipitation with calcite. They quantified the relation between phosphate in the solid phase with activities of Ca^{2+} and PO$_4{}^{3-}$ in the liquid phase. To obtain activities for lake water, we determined activity coefficients (γ) for Ca^{2+} (γ = 0.77) and PO$_4{}^{3-}$ ($\gamma = 2.9 \times 10^{-4}$) from the chemical equilibrium model WATEQ (Truesdale and Jones 1973) calibrated for the ionic content of Lake Michigan (Torrey 1976). By placing the average value of 33 μg$\cdot$L^{-1} for Ca^{2+}, we determined that for each microgram of P per litre, 1.56×10^{-4} μg P$\cdot$(g Ca^{2+})$^{-1}$ would coprecipitate.

Fecal pellet sinking rates — To calculate fecal pellet sinking rate and the effect of calcite content on sinking rate, we examined fecal pellets on oil-cleared slides of natural seston from control and experimental containers of the feeding experiments of Vanderploeg (1981a) before (May 31, 1978, and July 20, 1978) and during (September 22, 1978) the calcite whitings. Fecal pellet length, width, and calcite content as a percentage of pellet volume were recorded from a transect made on each of the slides to characterize the seston for another study (unpublished). Settling velocities were calculated using the semiempirical formula of Komar et al. (1981) for cylindrical fecal pellets for a water temperature of 7°C, the epilimnetic temperature during late spring and the upper hypolimnetic temperature during summer. In the case of pellets with only algae, the density was taken as 1.22 g$\cdot$cm^{-3} (e.g. Komar et al. 1981). To approximate the density of a fecal pellet containing

TABLE 3. Net feeding rate, NFR(±SE, $N = 4$), in the presence and absence of calcite. Treatment A (*Cyclotella* + HCl) corresponds to algal control without calcite; HCl was added just before Coulter analysis. Treatment B (*Cyclotella* + $CaCO_3$ + HCl) corresponds to experimental treatment with added calcite; HCl was added just before Coulter analysis. Treatment C (*Cyclotella* + calcite) is the same treatment (same animals and algae plus calcite suspension) as treatment B except the Coulter analyses to determine feeding rate in the bottles were done before HCl addition and analyses for treatment B. Normalized NFR is NFR predicted by linear extraplation for an algal concentration of $0.6\ m^3 \cdot L^{-1}$. Differences in NFR between treatments A and B show effects of calcite on NFR on algae. Differences in NFR between treatments B and C allow determination of whether calcite crystals are dissolved during gut passage. The comparisons are also shown by means of the ratios NFR(B)/NFR(A) and NFR(C)/NFR(B) where the letter in parentheses following the NFR labels the treatment. *Significantly different ($P < 0.05$, *t*-test) from treatment B; ** significantly different ($P < 0.01$, *t*-test) from treatment B.

Experiment No. and treatment	Particle concentration in suspension ($mm^3 \cdot L^{-1}$)			NFR [$10^7\ \mu m^3 \cdot (\text{mg dry wt})^{-1} \cdot h^{-1}$]		$\frac{NFR(B)}{NFR(A)}$	$\frac{NFR(C)}{NFR(B)}$
	Calcite	Algae	Total	Observed	Normalized		
1 A	0	0.60	0.60	1.78±0.10**	1.77		
B	0.60	0.72	1.32	0.907±0.078	0.904	0.510	1.15
C	0.60	0.72	1.32	1.04±0.20	1.04		
2 A	0	0.72	0.72	1.85±0.11*	1.55		
B	0.95	0.80	1.75	1.27±0.14	1.06	0.686	1.41
C	0.95	0.80	1.75	1.79±0.23	1.49		
3 A	0	0.57	0.57	1.39±0.035*	1.47		
B	0.56	0.68	1.25	1.18±0.056	1.25	0.849	0.949
C	0.56	0.68	1.25	1.12±0.15	1.19		
4 A	0	0.54	0.54	1.38±0.061*	1.53		
B	1.23	0.69	1.92	0.947±0.133	1.05	0.686	0.539
C	1.23	0.69	1.92	0.510±0.173	0.567		
5 A	0	0.49	0.49	1.30±0.06	1.58		
B	0.51	0.58	1.81	0.927±0.203	1.13	0.713	1.24
C	0.51	0.58	1.81	1.15±0.45	1.40		
6 A	0	0.54	0.54	1.09±0.04**	1.20		
B	0.51	0.59	1.10	1.37±0.05	1.51	1.26	1.16
C	0.51	0.59	1.10	1.59±0.06*	1.73		
7 A	0	0.74	0.74	2.33±0.16**	1.90		
B	0.85	0.83	1.68	0.547±0.369	0.446	0.235	0.832
C	0.85	0.83	1.68	0.455±0.377	0.371		
$\bar{X}$ ± SE of tr. A	0	0.60±0.04	0.60±0.04	1.59±0.16*	1.57±0.08**		
$\bar{X}$ ± SE of tr. B	0.85±0.11	0.70±0.04	1.55±0.12	1.02±0.10	1.05±0.12		
$\bar{X}$ ± SE of tr. C	0.85±0.11	0.70±0.04	1.55±0.12	1.09±0.19	1.11±0.19		
$\bar{X}$ ± SE of ratios						0.706±0.118	1.12±0.08

calcite, we took the weighted average (by percent volume) of calcite and algal densities. To approximate the density contribution of calcite to the pellet, we assumed that the calcite had a pore space volume of 30% (Wakeman 1975).

Results and Discussion

Zooplankton feeding

Experiments with *D. pulex* showed that calcite affected feeding rate on algae and that the feeding of *D. pulex* did not affect calcite concentration (Table 3). Results of the seven paired experiments are given in terms of NFR (net feeding rate = ingestion − egestion, Vanderploeg et al. 1984) on the algae in the algae-only treatment, NFR on the total assemblage of particles in the algae-plus-calcite treatment, and NFR on the algae in the algae-plus-calcite treatment. Trends in these data are also shown by ratios of NFR's for different treatments. NFR(B), the NFR on the algae in the algae-plus-calcite treatment, was less than NFR(A), the NFR on algae in the algae-only treatment, in six of the seven experiments; differences in five of these cases were significant. The mean overall NFR(B) for all seven experiments was significantly less than that of NFR(A), with the overall mean ratio NFR(B)/NFR(A) being 0.706. Mean overall NFR(C), the NFR on the calcite plus algae in the algae-plus-calcite treatment, was not significantly different from NFR(B), with the ratio NFR(C)/NFR(B) being 1.12. This result implies that little if any calcite was destroyed by its ingestion and subsequent gut passage.

Analysis of these same experiments by linear regression (Fig. 13) also showed that NFR on algae was signficantly decreased by calcite addition. At the average concentration of calcite found in epilimnion during September ($0.46 mm^3 \cdot L^{-1}$, Table 2), the regression predicts that feeding rate would drop by 16%.

Diaptomus sicilis' feeding was unaffected by the presence of a relatively high concentration of calcite in natural seston (Table 4). Clearance rates of the introduced alga for the total seston and the seston-minus-calcite treatments were not significantly different. The control experiment with treated and untreated mixtures of *Chlamydomonas* spp. showed that HCl addition followed by NaOH addition did not affect feeding rate.

Although the depression of NFR by increasing calcite concentration in *D. pulex* was modest, this depression is greater than that reported in previous studies with suspended bottom sediments or volcanic ash. The overall reduction in NFR as a

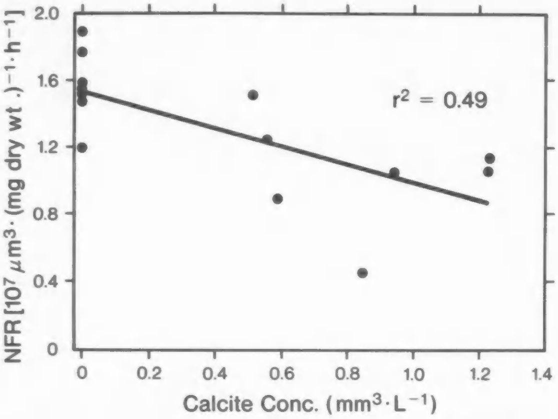

FIG. 13. Effect of calcite concentration on net feeding rate (NFR) of *Daphnia pulex* on *Cyclotella meneghiniana* offered at 0.8 $mm^3 \cdot L^{-1}$.

TABLE 4. Clearance rate (±SE, $N = 4$) of *Diaptomus sicilis* on radiolabeled *Cyclotella* sp. introduced into untreated seston and seston treated with a brief exposure to HCl followed by adjustment back to normal pH by NaOH addition.

Kind of seston and concentration ($mm^3 \cdot L^{-1}$)	Clearance rate ($mL \cdot animal^{-1} \cdot d^{-1}$) Untreated	Treated
Mixture of *Chlamydomonas* spp. (0.70)	10.04±1.00	12.94±1.34
Lake Michigan, Sept. 23, 1985: (calcite = 0.66; other = 0.75)	3.77±0.77	3.32±0.27

percent of the controls was ~30% in our experiments, having an average calcite concentration of 0.8mm$^3 \cdot L^{-1}$ (Table 3). Arruda et al. (1983, fig. 3) observed a 10% reduction in efficiency of incorporation of ^{14}C-labeled algae by *D pulex* feeding on suspended bottom sediment of 0.8mm $^3 \cdot L^{-1}$ (assuming density of sediment = 2.5 $g \cdot cm^{-3}$ to convert from the given units of weight concentration to our units of volume concentration); at 4 mm $^3 \cdot L^{-1}$ the reduction was 20%. However, they did observe reduction of 20% for *Daphnia parvula* over the concentration range of 0.08–0.8 mm $^3 \cdot L^{-1}$. Ingestion and assimilation rate of *D. pulex* were apparently unaffected by the presence of suspended volcancic ash over a concentration range of 4–100 mm $^3 \cdot L^{-1}$ (Gaddy and Parker 1986).

The smaller impact of suspended sediments and volcanic ash than calcite on assimilation of food by *D. pulex* could be a methodological artifact. Feeding conditions were most realistic in our study. In our study, *D. pulex* was prefed in algae or in algae plus calcite for 1 d. In the experiments of Arruda et al (1983), *D. pulex* fed for 1 h before the 1.5-h assimilation experiment with the ^{14}C-labeled alga was done. In the experiments of Gaddy and Parker (1986), zooplankton were starved for 15 h before introducing them into suspensions of the sediment and ^{14}C-labeled algae for the short-term (10 min) feeding experiment. In the study of Gaddy and Parker (1986), feeding rate on both algae and suspended sediment would have been very high because starved zooplankton introduced into algal suspensions feed at a much higher rate than animals acclimated to the suspension (e.g. Geller 1975). Although 1 h is usually considered long enough for acclimation to algal suspensions for feeding experiments with cladocerans (e.g. Geller 1975), there is no information on how long it takes for *Daphnia* or any other zooplankter to become acclimated to suspended inert particles. Possibly the 1-h acclimation period used by Arruda et al. (1983) was not long enough, or perhaps there are behavioral changes in the ability of cladocerans to reject inert suspensoids, by bolus rejection or by outwashing (Scavia et al. 1984), with time of exposure. Copepods are known to ingest suspensions of inert particles initially but reject them later (e.g. Paffenhöfer and Van Sant 1985). Perhaps there are physiological adaptations or injury, such as damage to the lining of the gut, that take time to occur in response to inert particles.

The greater reduction in feeding rate observed with calcite could result from its different size. The bottom sediment was composed of particles <10µm (qualitative microscopic observation), and the ash, which was allowed to settle for 72h undisturbed, was composed of particles 96% of which had volumes equivalent to less than 9 µm diameter. These descriptions suggest that the particles in these studies could have been much smaller than ours. The greater reduction of assimilation observed by Arruda et al. (1983) for *D parvula* than for *D. pulex* may reflect its smaller size (body length = 1.2 mm in *D. parvula* vs. 1.7 mm in *D. pulex*) relative to the diameter of ingested sediment.

Another possibility for the greater reduction in food consumption (NFR) with calcite is that calcite passage through the gut might neutralize the digestive juices and slow down gut passage or reduce assimilation efficiency. The digestive juices of crustaceans are slightly acidic, and many digestive enzymes function most efficiently under slightly acidic conditions (Vonk 1960).

The lack of a calcite effect on *Diaptomus*' feeding rate on natural seston is consistent with results of other investigations on the effects of inert suspensoids on *Diaptomus* and other copepods (Sherk et al. 1976; Paffenhöfer and Van Sant 1985; Gaddy and Parker 1986). It is difficult to draw firm conclusions from the studies of Sherk et al. (1976) and Gaddy and Parker (1986) because of potential methodological artifacts. In the study of Gaddy and Parker (1986), the same experimental design and ash concentrations were used for the copepod *Diaptomus ashlandi* as those for *D. pulex*. Sherk et al. (1976) used an experimental design similar to Gaddy and Parker's to evaluate the effects of sand of 6-µm median diameter on feeding rate of the estuarine copepods *Acartia tonsa* and *Eurytemora affinis* for sand concentrations above 20 mm $^3 \cdot L^{-1}$; respective reductions in feeding rate were 28–61 and 25–34% at 20 mm $^3 \cdot L^{-1}$. Experiments of Paffenhöfer and Van Sant (1985) are comparable because they acclimated their copepods for a long time (48–72 h) to the feeding conditions used in their feeding experiments with mixtures of 20-µm-diameter plastic microspheres and 12-µm-diameter algae. They found that over a microsphere concentration of 0–2.5 mm $^3 \cdot L^{-1}$, the microspheres had no effect on feeding rate of *Eucalanus pileatus* on the algae, presented at a concentration of 0.3 mm $^3 \cdot L^{-1}$.

Our results for *D. sicilis* and Paffenhöfer and Van Sant's(1985) results for *E. pileatus* suggest that copepod feeding will be unaffected by the concentration of calcite found in Lake Michigan. That *D. pulex* was affected and *D. sicilis* was not, is related to differences in feeding mechanisms between copepods and cladocerans. As noted above, cladocerans have limited ability to reject already filtered particles, while *Diaptomus* and other copeods like *Eucalanus* use long-range olfaction in conjunction with coordinated movements of the mouthparts to actively capture large food particles (e.g. Price et al. 1983; Paffenhöfer and Van Sant 1985; Vanderploeg and Paffenhöfer 1985), thereby increasing the probability of capturing food particles relative to inert particles, which are captured mostly by the filtering mode of feeding (Paffenhöfer and Van Sant 1985). Whether a group of passively captured (filtered) particles is swallowed or rejected as a group in copepods (Paffenhöfer and Van Sant 1985) or cladocerans depends on the composite taste of the group. The great sensitivity of *Diaptomus* to discriminate between small (6-μm-diameter) algae, flavored microspheres, and unflavored microspheres (DeMott 1986) suggests that even this group-taste selection may be more sensitive to food quality in *Diaptomus* than in cladocerans.

Some caution must be noted in our conclusions that *Diaptomus* is unaffected by the calcite concentration found in Lake Michigan. Neither our study nor that of Paffenhöfer and Van Sant (1985) rules out the possibility of long-term physiological effects of calcite ingestion. Moreover, *Diaptomus*, being a small-particle specialist compared with *E. pileatus* (Vanderploeg and Paffenhöfer 1985), will probably ingest more inert food particles than *E. pileatus* because the filter-feeding mode is a more important contributer to the total feeding of *D. sicilis* than in *E. pileatus*. The large proportion of calcite in the fecal pellets of *D. sicilis* shows that significant quantities of calcite are ingested despite *Diaptomus*' ability to discriminate between particles on the basis of taste. It must be remembered, however, that digestion of algae will increase the proportion of calcite found in the pellet relative to the proportion actually ingested.

Optical effects of calcite

A significant increase in light scattering by calcite could diminish primary production by decreasing light available in the water column for photosynthesis. Figure 14 predicts the effect of a calcite concentration of 1 $mm^3 \cdot L^{-1}$ in each size category using anomolous diffraction theory (van de Hulst 1957; Kirk 1983, 1985). The light scattering coefficient, b, peaks at 1 μm and decreases rapidly thereafter with increasing particle diameter. Because the peak in the volume spectrum of calcite is located at 7 μm (Fig. 10), the efficiency of light scattering by calcite in Lake Michigan will be relatively low (Fig. 14). Figure 14 also shows b for 1 $mm^3 \cdot L^{-1}$ total concentration of calcite having the average volume spectrum in Fig. 10. Most of the scattering centers on particles between 2 and 10 μm. Calcite in Lake Michigan at $1 mm^3 \cdot L^{-1}$ gives $b = 0.48 \cdot m^{-1}$; at 0.69 $mm^3 \cdot L^{-1}$, the highest calcite concentration observed, $b = 0.48 \times 0.69 = 0.33 \cdot m^{-1}$. As far as scattering coefficients for inland waters are concerned, a value of $0.33 \cdot m^{-1}$ is relatively low, since b values for inland waters typically range between 1 and $10 \cdot m^{-1}$ (Kirk 1985).

If the absorption coefficient, a, for Lake Michigan water were known, the effect of calcite on the vertical attenuation coefficient, K_d, of light extinction could be predicted (Kirk 1983, 1985). Since a is not known, we cannot make this prediction. However, measurements of K_d before during and after whitings suggest that the effect of calcite on K_d is small. Ladewski and Stoermer (1973) observed a 10% increase in K_d during September whitings in 1971 and 1972. A similar modest effect of calcite is suggested by the K_d values reported by Scavia et al. (1986), who observed an increase in K_d from $0.17–0.20 \cdot m^{-1}$ during July 1984 to $0.23 \cdot m^{-1}$ during a whiting on August 24, 1984.

In contrast with K_d, Secchi depth is greatly affected by the calcite. A 65% drop in Secchi depth is typically seen, with prewhiting values of about 12 m and whiting values of about 5 m (Ladewski and Stoermer 1973). Scavia et al. (1986) also showed this systematic decrease in Secchi depth occurring regularly over the 10-yr period 1975–84, except during 1983. This drop in Secchi depth is caused by irradiance upwelling from backscattering from the calcite particles (Ladewski and Stoermer 1973; Kirk 1985). Thus, the Secchi depth is a sensitive monitor of the whiting phenomenon and poor predictor of K_d. This follows because Secchi depth decreases both with increasing K_d and irradiance upwelling (Ladewski and Stoermer 1973; Kirk 1985).

Calcite and nutrient dynamics

The weight-averaged sinking rate of calcite having the particle-size spectrum shown in Fig. 10 is $5.7 \ m \cdot d^{-1}$. For a 20-m-deep epilimnion, the fraction of calcite lost is $0.29 \cdot d^{-1}$. For the average calcite concentration of $0.46 \ mm \cdot L^{-1}$ (1.25 $mg \cdot L^{-1}$) for September during whiting years (Table 2), the downward flux of calcite is $7.2 \ g \cdot m^{-2} \cdot d^{-1}$ and the fraction of phosphate removed from the epilimnion is $0.056 \cdot d^{-1}$, which implies a turnover time of 18 d for inorganic P removed by coprecipitation and sinking.

At first glance this estimate of inorganic P removal would appear to represent a significant loss of P available to sustain phytoplankton growth in late summer, since the whiting period lasts for 1 to 1.5 mo. However, Rigler bioassay estimates of P available to phytoplankton during 1976, a whiting year, do not show any marked decrease during late August and September (Tarapchak and Rubitschun 1981). The probable reason for this apparent discrepancy is that concentration of inorganic P available to phytoplankton for growth and to calcite for coprecipitation and sinking from the epilimnion is well buffered by its rapid exchange with the large nonavailable pool of P in the system that only slowly becomes inorganic P. Rigler estimates of inorganic P vary between 0.6 and 0.8 $\mu g \cdot L^{-1}$ in the epilimnion of Lake Michigan during late summer (Tarapchak and Rubitschun 1981). In contrast, dissolved total P concentration is about 5 $\mu g \cdot L^{-1}$ (Bartone and Schelske 1982), and there is also P associated with detritus, living algae, and zooplankton. Turnover of the inorganic P pool is <30 min (Fahnenstiel and Scavia 1987), and its size is largely regulated by the balance between the loss process of phytoplankton uptake and return processes such as zooplankton grazing, phytoplankton excretion of organic P with subsequent bacterial breakdown to inorganic P, and bacterial breakdown of detritus (e.g. Scavia 1979). Although coprecipitation may remove about 6% of the inorganic P per day, this is only a small fraction of the total P pool.

Fecal pellet sinking rate

Fecal pellets in the water column are quite small, and when they contain remains of algae and other organic seston, they have a low sinking rate, the mean value being approximately 5 $m \cdot d^{-1}$ (Table 5). During September, calcite (68% of pellet volume (Table 5)) increases density and sinking rate by a factor of 4 to give $20 \ m \cdot d^{-1}$. A sinking rate of $5 \ m \cdot d^{-1}$ in a 20-m-deep epilimnion implies a pellet turnover time of 4 d. This should be sufficient time for significant bacterial decay to occur (e.g. Turner and Ferrante 1979), with subsequent recycling of P, N, and Si within the epilimnion. Increasing fecal pellet sinking by a factor of 4, by calcite inclusion, may remove pellets quickly enough to greatly diminish epilimnetic recycling of fecal-bound

TABLE 5. Size ± SE, calcite content, and calculated settling velocities (Komar et al. 1981) of fecal pellets before and during calcite whiting. Settling velocities calculated without and with consideration of the pellets' added calcite mass are indicated as "−calcite" and "+calcite."

Date	N	Length (μm)	Width (μm)	Calcite content (% by volume)	Settling velocity $(m \cdot d^{-1})^a$ −calcite	Settling velocity $(m \cdot d^{-1})^a$ +calcite
May 20, 1978	22	43.7±8.2	24.1±4.1	0.0	7.3	7.3
July 25, 1978	6	28.5±6.3	14.5±2.6	0.0	2.7	2.7
September 20, 1978	18	40.1±10.4	20.0±3.6	68.3	5.2	21.2

[a] Calculated for 7°C.

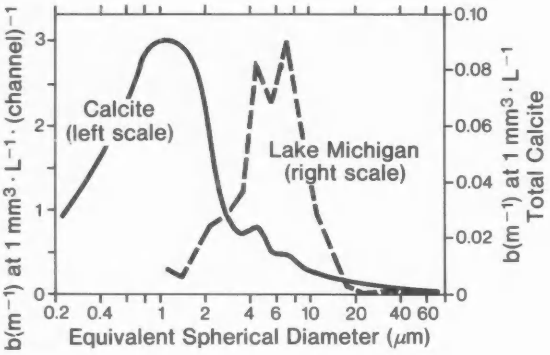

FIG. 14. Effect of calcite size on the light scattering coefficient, *b* (left scale), and the effect on *b* of calcite having a total concentration of 1 $mm^3 \cdot L^{-1}$ and particle-size spectrum shape like that of Fig. 10 (right scale).

nutrients. Because the majority of the epilimnetic zooplankton has been cladocerans during the late summer of recent years (e.g. Evans and Jude 1986), much of the zooplankton feces will not be in the form of fecal pellets packaged neatly by a peritrophic membrane. However, cladocerans do produce coherent fecal masses in suspensions of mineral particles (Zurek 1982; Gliwicz 1986). Calcite inclusion will likewise increase sinking rate of cladoceran fecal material. Owing to the filtering mode of feeding of most cladocerans, fecal material would expected to be even more rich in calcite than in copepod fecal pellets.

A maximum estimate of the effect of calcite on cycling of fecal-bound nutrients may be obtained by assuming that all fecal pellets produced during the calcite whiting period sink out of the epilimnion before they decompose. Clearance rates on phytoplankton in the epilimnion of Lake Michigan should be on the order of $0.1 \cdot d^{-1}$ (Scavia 1980; Scavia and Fahnenstiel 1987). Of the P ingested, 23% should be excreted in fecal pellets and 60% as soluble P (Butler et al. 1970). Thus, the fraction of phytoplankton-bound P lost by fecal pellet sinking is $0.1 \cdot d^{-1} \times 0.23 = 0.023 \cdot d^{-1}$. Over the period of a month, this would imply a loss of 50% of the amount of P associated with the phytoplankton P pool. By September, Si has long been limiting to diatoms. Since all ingested Si appears in the fecal pellets, $0.1 \cdot d^{-1}$ will be cleared out of the epilimnion. If Si were not already limiting, the calcite whitings could accelerate this loss. As we have seen above, Rigler estimates of orthophosphate did not change greatly during the whiting period. Again this would imply that calcite whitings do not control P cycling in the epilimnion. Also, total P and total dissolved P do not decrease dramatically during September (Bartone and Schelske 1982). However, this is not to say that the losses from coprecipitation and accelerated fecal sinking have no effects. Perhaps in the absence of these forces, orthophosphate concentrations would be higher, that is, increase during September in the absence of whitings. We cannot draw more definitive conclusions because pool sizes and fluxes between various P components have not as yet been worked out for Lake Michigan.

General Discussion and Conclusion

Little experimental work on the effect of calcite on processes important to plankton dynamics had been done previously. Our approach in this study has been to first characterize the calcite in terms of its contribution to the particle-size spectrum. This characterization has allowed us to explore potentially important ecological effects with the aid of experimentation and mathematical analysis, since particle size and abundance of calcite are key factors for predicting downward flux of calcite and effects of calcite on light extinction and zooplankton grazing.

Our experimental work and analyses suggest that food web dynamics are more controlling of rather than controlled by calcite whitings. Of particular interest was the absence of a whiting even in 1983 that we attributed to predation by salmon, the top carnivore in the system. This is the first example of top-down control of the calcite system of which we are aware. Bottom-up control of the system has been reported for the previously oligotrophic Lake Breiter Lucin (Koschel et al. 1983). Increased P loading to this lake from cultural eutrophication caused increased CO_2 uptake by algae, thus precipitating the whitings. Obviously, any factor affecting primary production will affect calcite whitings.

Our conclusion that the plankton is only modestly affected by calcite whitings in Lake Michigan does not necessarily apply to other lakes, especially those more eutrophic or those having greater calcium hardness than Lake Michigan. For example, light extinction may be significantly increased during whitings (e.g. Koschel et al. 1983; Weidemann et al. 1985) in certain mesotrophic lakes. Also, total dissolved P and total P decreased dramatically during the whiting in mesotrophic Lake Breiter Lucin (G.D.R.) (Koschel et al. 1983), but apparently not in Lake Michigan (Bartone and Schelske 1982, fig. 10b). Obviously, the intensity of the whiting is an important factor. Another factor may be timing. In contrast with Lake Michigan and other Great Lakes, calcite whitings typically occur from late spring to midsummer (Kelts and Hsu 1978; Koschel et al. 1983). We noted the potentially large loss of particulate P and Si from fecal sinking during September in Lake Michigan. If this loss occurred during June, at the beginning of stratification, rather than near the end during September, significant impacts to nutrient cycling and plankton dynamics might have been observed.

Further insight into the importance of the various ecological effects of calcite can be gained by comparing our results with those of Koschel et al. (1983) on Lake Breiter Lucin. Their study was designed to test the importance of the three calcite effects enumerated above. This was done by standard limnological measurements coupled with simulation analysis. In Lake Breiter Lucin, dissolved and total P, phytoplankton biomass, and light transmission all decreased during the whiting period. Their simulation analyses suggested that increased sinking of phytoplankton from flocculation with calcite was the most important of the calcite-effects mechanisms they hypothesized. The analyses were done by arbitrarily increasing the sinking rate of the algae. Empirical evidence cited for flocculation was increased organic C flux to sediment traps during the whiting period. We note that the mechanism of increased sinking rate of fecal material is consistent with the observed increased organic C flux and possibly with their simulation results, since increased fecal sinking and algal sinking can lead to similar results, especially for dissolved and total P. Also, examination of the same slides used for fecal pellet analyses showed no obvious evidence of calcite flocculation with or encrustation on intact algae. All calcite-algal aggregates appeared to be fecal pellets or matter.

Our conclusion that orthophosphate, as estimated by Rigler's bioassay, was unaffected by the whitings in Lake Michigan parallels the conclusion of Koschel et al. (1983) in that orthophosphate, measured as soluble reactive phosphorus (SRP), did not change in Lake Breiter Lucin. However, since SRP is poor measure of orthophosphate (e.g. Tarapchak and Rubitschun 1981; Tarapchak et al. 1982), we cannot be certain how orthophosphate behaved in Lake Breiter Lucin. As in Lake Michigan, orthophosphate concentration will be buffered by its rapid exchange with the large nonavailable pool of P in the system. Perhaps, its concentration in Lake Breiter Lucin is also relatively unaffected.

For the concentration of calcite found in Lake Michigan, we concluded that feeding in cladocerans was only modestly depressed (16%), and *Diaptomus* was unaffected. For 0.8 mm^3 calcite$\cdot L^{-1}$, the drop in feeding rate was 30%, a value high enough to expect a significant decrease in reproduction. In Lake Breiter Lucin, calcite concentrations during the peak of the whitings ranged between 1.8 and 5.5 $mm^3 \cdot L^{-1}$. At these concentrations, large effects on cladoceran populations would be expected. In addition to lowered feeding, calcite would slightly increase respiration, owing to increased swimming effort to overcome the weight of mineral particles in the gut (Zurek 1982). With concentrations of calcite as high as those in Lake Breiter Lucin, cladocerans may be put at a significant disadvantage relative to copepods. Of all the potential effects of calcite whitings, perhaps calcite–zooplankton interactions are the most deserving of further research.

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Comparison of Acoustic Reflections from Spherical Objects and Fish Using a Dual-Beam Echosounder

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Dickie, L. M., and P. R. Boudreau. 1987. Comparison of acoustic reflections from spherical objects and fish using a dual-beam echosounder. Can. J. Fish. Aquat. Sci. 44: 1915–1921.

Comparison of dual-beam echos from spherical targets and from fish demonstrates that the fish directivity pattern causes significant distortion and enhanced variance in acoustic measurements related to abundance estimates. Dual-beam data on single fish targets in the laboratory and field may be used to define a correction which more accurately describes the observed or field target strengths of fish, permitting a more precise estimate of the associated measures of size, density, and biomass.

La comparaison des échos produits par un échosondeur à deux faisceaux et réfléchis par des cibles sphériques et des poissons indique que l'orientation des poissons est un facteur important de distorsion et de variance dans les mesures acoustiques servant aux estimations de l'abondance. Les mesures prises avec ce genre d'échosondeur sur le terrain et en laboratoire avec des cibles constituées d'un seul poisson peuvent être utilisées pour déterminer la correction à apporter qui décrit le mieux la force du signal du poisson observé sur le terrain, afin d'obtenir des estimations plus exactes de la grosseur, de la densité et de la biomasse.

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The accuracy and precision of estimates of the average target strength (TS) of fish directly affect acoustic determination of fish density or abundance. In earlier papers (Dickie et al. 1983, 1984) we have described the relative success of attempts to use the ECOLOG dual-beam acoustic system to estimate the density distribution of demersal fish of different sizes along survey transects, implying potentially high precision in TS determination. In this paper we describe a series of laboratory and field observations which appear to require consideration of a previously unappreciated source of systematic distortion in the measured fish TS. Evidence for this distortion is obtained from comparative study of changes in reflective power of physical targets and fish with perspective in the acoustic beam. From the comparison we conclude that our observations reflect actual acoustic properties of fish targets, probably involving interaction of fish directivity with changing position in the transducer beam. In this paper we give detailed acoustic measurements of various single targets, including moving fish targets under laboratory and field conditions. We conclude that there is a systematic trajectory of echo levels which characterizes repeated acoustic measurements of moving fish targets, and while we demonstrate it only for single- and dual-beam acoustic systems, from its appearance as a property of living fish as targets, it must also affect measurements of TS in split-beam systems. The results of our experience to date suggest an appropriate means of using dual-beam data to correct for the effect and to get improved estimates of fish size and density under field conditions.

Equipment and Methods

The ECOLOG fisheries acoustic system has been described in the earlier papers. It employs a towed, vertically oriented dual-beam transducer in which the two nested components have half-power half-angles of 3.25° on the narrow beam and 13° on the wide beam. The system operates at 50 kHz with 0.4-ms (0.3-m) pulses generated by a 5-kW power amplifier at 125 pulses/min. A Simrad EK 50 echo-sounder is used as the pulse generator; signals are transmitted on the narrow beam. Echoes received by both transducers are amplified by a specially built TVG receiver. The received, demodulated signals are sampled by a microprocessor unit at 0.1-ms intervals (10 kHz or 7.5 cm) for amplitude and time after transmission. Each such sample is referred to here as an "interrupt." All interrupts above a preset threshold from each transducer are digitized and recorded separately on magnetic tape throughout each pulse, up to and including the bottom echo. "On line" samples of acoustic data are analyzed aboard ship to help control the sampling program. The digitized full data set is readily available for detailed analysis at the end of the cruise or experiment.

In field application the ECOLOG transducer is mounted in a dead weight towed body and suspended from the side of the survey vessel on an hydraulic articulated crane. The towing cable is spooled on an hydraulic winch and connected to the deck instruments through a specially constructed slip-ring. While the transducer can be towed at 10–12 kn (1 kn = 1.85 km/h) at a depth of between 20 and 30 m below the surface, it is stable at speeds as low as 2 kn and can be towed deeper at slower speeds. Some of the field acoustic data presented here are for transects where acoustic and trawl net sampling were carried out simultaneously at 3.5 kn. The pairing of acoustic and net samples allows species identification and a comparison of estimates of population size structure and density. In addition we show here data collected for physical targets and fish when the ship was stationary at dockside or at anchor. In these cases the transducer body was lowered over the side of the vessel with a horizontal framework of four metal arms of approximately 200 cm length each, bolted in a cross along the midline and across the axis of the body. Different targets were suspended at various positions under the transducer by adjustable monofilament lines running from the ends of the arms.

Interpretation of the field data is supported by observations of single fish and more or less spherical objects in laboratory settings. For such observations the transducer was suspended in the tank just below the water surface. Fish targets were measured as the fish swam normally at various ranges through the acoustic beam (Dickie et al. 1984). Inanimate objects were moved through the beam on monofilament lines.

In the field we have usually used a 0.7-V threshold. Signals falling below this threshold are not processed by the microprocessor. This threshold level corresponds to an approximately 8-cm cod on axis and is adopted as a way of maintaining a signal to noise ratio greater than 11 dB for a threshold signal. At our usual operating depths of 80–100 m the maximum noise levels are of the order of 0.1 and 0.2 V on the narrow and wide transducers, respectively.

In general, in both laboratory and field situations, above-threshold signals from a single fish are returned in several interrupts within a pulse and in several successive pulse transmissions. These acoustic samples are used to define a pulse envelope for a single fish. Within a given pulse, our processing system ordinarily chooses each peak value separated by >0.2 ms from any other peak as the signal strength of a fish in that pulse. Over a number of pulses, depending on range, ship speed, and fish size, the ECOLOG transmission rate of 125 pulses/min affords a calculable maximum possible number of successive transmissions in which a single fish can be detected. The peak samples from successive pulses are defined as belonging to the same fish if they are within a 0.2-ms range of a peak in the previous pulse and do not exceed the specified maximum number. In the field data the series of successive samples is used in the development of the TS corrections described in the next section. To avoid any question of ambiguities due to overlapping targets, in the specific comparative studies of physical and fish targets reported here, data defined to be single fish have been restricted to records of distinctly separate targets, that is, echo envelopes separated from others by 0.2 ms within all the pulses used. Measured peak voltages are converted to RMS values squared and expressed as TS (decibels).

In the data presented here, physical targets have been studied in field conditions at ranges as close as 6.5 m and as far away as 17 m. They are compared here with fish targets at dockside at ranges as close as 10 m and in ordinary field operations at ranges of the order of 70–80 m, although observations are routinely made at ranges as great as 200 m. In the laboratory we have observed both physical and fish targets with similar variances around the measured TS at ranges as little as 3 m. This is in accordance with expectations of "far field" measurements. According to Urick (1975) the far field would be expected to begin at ranges greater than about 2.5 m.

Dual-Beam Theory

The squared voltage or power (E^2) returned from a sound pulse by a given target, after appropriate amplification through the TVG to correct for spreading and absorption losses in water, has been described by the equation

$$(1) \quad E^2 = kb_t(\theta, \phi)b_r(\theta, \phi)\sigma_{bs}$$

where k is the system scaling factor, $b_t(\theta, \phi)$ is the beam pattern of the transmitted pulse, $b_r(\theta, \phi)$ is the beam pattern of the received pulse, and σ_{bs} is the backscattering cross section of the target.

Traynor and Ehrenberg (1979) suggested that if two nested transducers of different beam widths are used and the signal is transmitted on the narrow beam and received on both narrow and wide, two equations may be written:

$$(2) \quad E_n^2 = k_n b_n^2(\theta, \phi)\sigma_{bs}$$

$$(3) \quad E_w^2 = k_w b_n(\theta, \phi) b_w(\theta, \phi)\sigma_{bs}$$

where subscripts n and w refer to the narrow and wide beams, respectively. The ratio of the power received on the two transducers is

$$(4) \quad \frac{E_n^2}{E_w^2} = \frac{k_n}{k_w} \times \frac{b_n(\theta, \phi)}{b_w(\theta, \phi)}.$$

If we solve for σ_{bs} from eq. (3) for the wide beam,

$$(5) \quad \sigma_{bs} = \frac{E_w^2}{k_w b_n(\theta, \phi) b_w(\theta, \phi)},$$

and for $b_n(\theta, \phi)$ from eq. (4),

$$(6) \quad b_n(\theta, \phi) = \frac{k_w}{k_n} \times b_w(\theta, \phi) \times \frac{E_n^2}{E_w^2},$$

then by substitution of (6) in (5):

$$(7) \quad \sigma_{bs} = \frac{k_n}{k_w} \times \frac{1}{k_w} \times \frac{1}{b_w^2(\theta, \phi)} \times \frac{E_w^2}{E_n^2} \times E_w^2.$$

As evident from eq. (7), k_n/k_w scales the two transducers to the same relative terms and k_w is the general scaling factor, $1/b_w^2(\theta, \phi)$ is the directivity index for angular scaling of the wide beam, the ratio term E_w^2/E_n^2 is an index of the radial distance of the target from the common axis, and E_w^2 is the power received on the wide-beam transducer.

Traynor and Ehrenberg (1979) noted that if the calibration scales are set such that $k_n = k_w$ and the wide beam has been constructed such that $b_w^2(\theta, \phi) = 1$ over the range of the narrow beam, then eq. (7) reduces to

$$(8) \quad \sigma_{bs} = \frac{1}{k_w} \times \frac{E_w^4}{E_n^2}.$$

In calibration, the gains and calibration constants are set so that in effect the value of the constant k_w is absorbed in the readings of echo level; that is, we redefine echo level (EL) as $E^2 \times k$. The equation may then be rewritten in the customary decibel form as

$$(9) \quad \mathrm{TS} = 10 \log \sigma_{bs} = 2(\mathrm{EL}_w) - \mathrm{EL}_n$$

where $\mathrm{EL}_w = 10 \log E_w^2 \times k_w$ and $\mathrm{EL}_n = 10 \log E_n^2 \times k_n$. This form, called here the (2W–N) correction, makes clear the conclusion that given appropriate conditions, the TS of an ideal target can be calculated from the dual-beam system at any position in the beam as the constant difference between the echo level measurements derived from each of the transducers. Data in the form of eq. (8) and (9) were used to analyze dual-beam records in Dickie et al. (1984).

If a moving single target is observed repeatedly in an experiment or in the field, then σ_{bs} at different positions in this dual-beam system (or the product $k_w\sigma_{\mathrm{bs}}$) will be constant within experimental error. That is, we may rewrite eq. (9) in the form

$$(10) \quad 10 \log \mathrm{EL}_w = \text{constant} + a(10 \log \mathrm{EL}_n)$$

where the constant is proportional to 10 log (σ_{bs}). It follows from eq. (9) that $a = 0.5$ for an ideal target. The equation in this form emphasizes the fact that for points at various distances off the axis of the dual-beam sonified cone, the graph of echo level of the wide-beam signal plotted against echo level of the narrow will be a line with a slope $a = 0.5$. At the point of intersection of this line with the 1:1 line, where the two transducers receive equal reflections from the target, $\mathrm{EL}_w = \mathrm{EL}_n = 10 \log \sigma_{\mathrm{bs}} = \mathrm{TS}$. That is, the intersection of the graph of echo levels with the 1:1 line is an estimate of the on-axis TS of the object. In the analysis described below, we make use of eq. (10) for ideal spherical objects as empirical tests of the performance of the acoustic system. If targets are large relative to acoustic wavelength, their reflections are in the geometric range, that is, since fish observed in our studies are >10 cm body length, and wavelength of the 50-kHz pulse is 3 cm, their reflection will be "directional." We suggest in what follows that deviations from the simple expectations of eq. (10) provide a useful means of characterizing the reflective properties of moving non-ideal targets, such as living fish.

Results

Acoustic Reflection from Spherical Targets

We have used the ECOLOG system to study TS of a number of more or less regular spherical objects, including an industrial grade light bulb, oblong balloons of various sizes, and solid metallic spheres. The light bulb was used in experiments to illustrate the beam patterns of the two transducers, the effects of beam angle, transmitted voltages, and the effects of apparent TS on the numbers of samples taken at the 0.1-ms sampling rate of the system. Results as described in Dickie et al. (1983) indicated satisfactory system performance and are not repeated here. In this report, we consider measurements of the balloons that were observed in experimental tanks where we also have observations of normally swimming single fish, and of two solid spheres that we have used as field reference targets and that are comparable with reference targets used in European laboratories to calibrate fisheries acoustic systems (Foote and MacLennan 1984).

In Fig. 1, data for the two solid metallic spheres illustrate the performance of the dual-beam system. The larger sphere is a stainless steel ball of 0.06 m diameter formerly advocated by the Simrad Company as a calibration sphere for their EK-50 sounder. A target like it has been studied in detail by MacLennan (1981) and perceived to have a TS of −35.5 dB at 38 kHz. Using his calculations for a 50-kHz system, our sphere should have a TS of approximately −34.2 kHz. We have measured it in two different situations: at anchor in the field at Bonne Bay, Newfoundland, and at dockside at the Bedford Institute of Oceanography (BIO). The second sphere is a solid copper ball of 0.045 m diameter advocated as a reference sphere by Foote (1982). This sphere was purchased from the Simrad Company of Norway and calculated by K. G. Foote (pers. comm.) to have a TS = −36.2 dB at a frequency of 50 kHz and a pulse length of 0.3 m. We measured it at dockside at BIO.

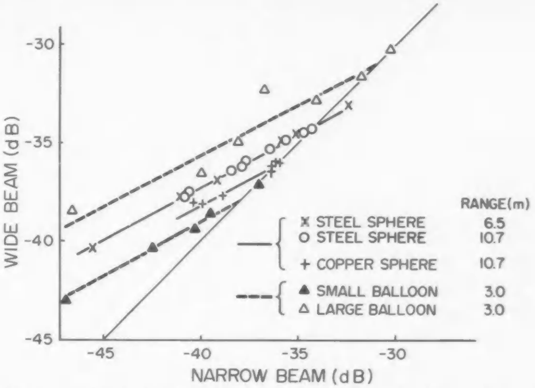

FIG. 1. Relationship between received echo level of the wide and narrow beams for spherical physical targets at different positions in the beam. Empirical linear regression lines are shown for each target. See Table 1 for empirical values of slopes.

Figure 1 shows observations of these physical targets at different ranges and positions in the beam, plotted in the form of eq. (10). The stainless steel sphere was observed at three ranges: 7, 11, and 17 m from the transducer. Measurement data for the two closer ranges are shown as individual points and the indicated single line of best fit calculated and drawn. Comparable data for the copper sphere are given for one range. Each point on either line is the average of more than 200 acoustic samples taken at a given beam position and measurement occasion. Standard errors of measurement for each such measurement occasion are of the order of 0.01 dB and are smaller than the diameter of the individual points plotted on the diagram. In this sense it may be said that the accuracy of measurement on a given measurement occasion is of the order of ±0.01 dB.

Different points along the line for a given target represent a series of measurements taken at different positions in the beam. Small variations about the line are associated with the movement of the targets from one position to another in the beam. Data have been studied for the effects of a variety of different manipulations on the variance about the mean measured TS including movement of the sphere in range and position in the beam, movement of the transducer and suspension system, and replicate occasions of turning the ECOLOG system on and off. The results suggest that with a standard target, an overall accuracy of measurement within ±0.5 dB was attainable. That is, normal variance in the state of the many electric, electronic, and physical systems allows an agreement between successive uses of the acoustic system of within 1 dB with a target that is ideally measurable to within ±0.1 dB (Foote 1982).

The graphs of echo levels of both the copper and stainless steel spheres at various positions in the beam show relatively small variations about the lines. The intersection of the

TABLE 1. Mean and standard deviation of echo level data presented in Fig. 1, 2, and 4. Data for spherical objects are given for wide beam, narrow beam, and (2W–N). For fish, additional data are given for TS after the removal of an average empirical $a = 0.75$. Values of empirical slope for each target are presented in the right-hand column. Summary rows show the grand means for the targets as well as the average of their standard deviations.

Target		Wide beam	Narrow beam	2W–N	Corrected TS	Empirical slopes (a)
Steel sphere	Mean	−36.0	−37.7	−34.3		0.54
	SD	1.82	3.38	0.31		
Copper sphere	Mean	−36.7	−37.2	−36.2		0.50
	SD	0.90	1.75	0.35		
Small balloon	Mean	−39.1	−41.4	−36.8		0.54
	SD	2.62	4.79	0.57		
Large balloon	Mean	−35.0	−39.1	−31.0		0.51
	SD	4.44	8.41	2.08		
Mean of targets		−36.7	−38.9	−34.6		
Average SD		2.45	4.57	0.83		
Fig. 2 fish	Mean	−32.4	−35.4	−33.1	−37.6	0.79
	SD	4.14	5.08	3.63	2.29	
Fig. 4 fish						
No. 1	Mean	−35.9	−39.6	−32.1	−34.3	0.58
	SD	2.18	2.75	3.02	3.34	
No. 2	Mean	−35.2	−36.5	−34.0	−37.9	0.69
	SD	3.15	−4.20	2.95	2.69	
No. 3	Mean	−36.3	−37.6	−35.9	−38.4	0.82
	SD	4.93	5.98	2.67	1.65	
No. 4	Mean	−37.1	−37.8	−36.4	−39.8	0.91
	SD	4.65	5.10	4.22	1.83	
Mean of fish		−35.7	−37.4	−34.3	−37.4	0.75
Average SD		3.81	4.62	3.30	2.36	

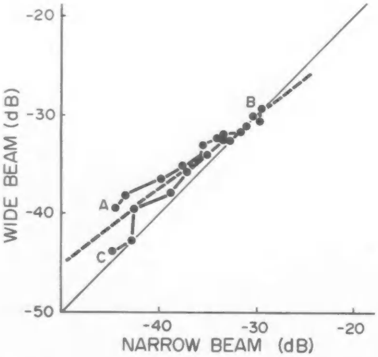

FIG. 2. Trajectory of received echo level of the wide and narrow beams for a 37-cm fish swimming through the acoustic beam. A, Fish entering beam; B, fish in near axial position; C, fish exiting beam. The broken line is the overall linear regression.

regression equation calculated as eq. (10) at its intersection with the 1:1 diagonal construction line (Fig. 1) can be estimated with considerable statistical accuracy, indicating that the projection to the diagonal provides a precise measure of on-axis TS. Data in Table 1, which are discussed in more detail below, show the reduction in variance which results from the calculation based on the (2W–N) algorithm. Figure 1 and Table 1 also show that, as expected from eq. (10), dual-beam plots of echo levels from the two transducers for each of the two standard reference spheres have a common slope $a = 0.5$.

On Fig. 1 we also show dual-beam data for two oblong balloons observed in tanks of seawater at an on-axis range of 3.0 m. The balloons, in air, measured approximately 10 and 15 cm in length. They were wrapped in a single layer of fine-mesh monofilament netting from which was suspended a lead weight on a line of about 50 cm in length. They were lowered to the bottom of the tank and moved about under the transducer to various points by monofilament lines attached to the weights. That is, the balloons were observed at a range of about 3.0 at 0.5 m off bottom. The suspension system of the balloons allowed them to rotate on their anchorage points and there was also some horizontal movement due to gentle tank currents. Their dimensions under pressure were not measured, but they appeared from the surface to maintain their general shape. The plotted data show that these oblong targets, observed from above, but with various orientations and positions in the beam, yield graphs of 0.5 slope with intersections on the 1:1 diagonal that have a slightly lower precision than was afforded by the metal spheres. However, their common slope is parallel to that seen with the solid reference spheres. The difference between their respective points of intersection with the 1:1 diagonal reflects differences expected from their different backscattering cross-sections.

Acoustic Reflection from Fish Targets

In Fig. 2, we illustrate the results from acoustic dual-beam measurement of a single fish of approximately 37 cm in length.

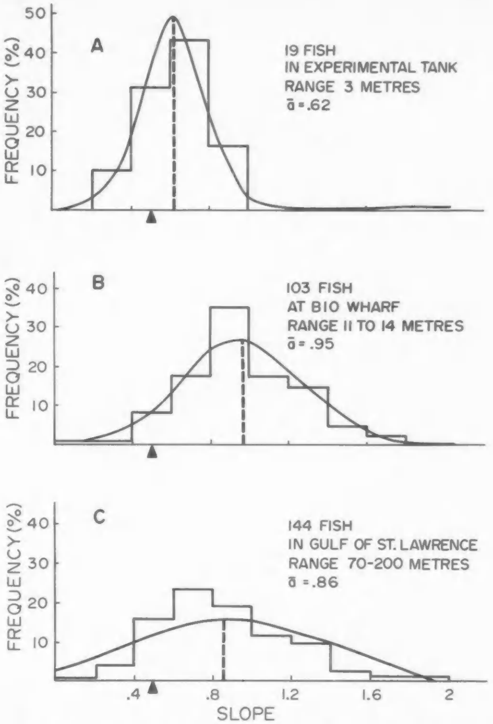

FIG. 3. Frequency distributions of empirical slopes a (eq. (10)) calculated from data on individual free-swimming fish. (A) 19 observations of a 37-cm fish in the pool tank; (B) 103 observations of fish at the BIO wharf; (C) 144 observations of fish during survey transects. Broken lines indicate means. Curves are normal distributions for the empirical means and standard deviations. An arrowhead on the abcissa indicates theoretically expected slope $a = 0.5$.

This fish was observed in the large tank in which we made observations of the balloons illustrated in Fig. 1. The fish swam at a range of about 3 m in a normal leisurely fashion through the centre of the acoustic beam, the position of which had been visually marked on the floor of the tank (range of 3.5 m) with metallic rings. The fish was observed simultaneously from above by the transducer floated at the surface and by a television camera mounted at the side of the transducer. The experimental set-up was as described in Dickie et al. (1984).

The trajectory of the successive observations of echo level (Fig. 2) is characteristic of acoustic reflections from fish at various ranges from the transducer. In this instance, as the fish swam into the beam an above-threshold signal was recorded on the wide and narrow beams with point "A" EL coordinates. As it moved towards the centre of the beam, there is a more or less linearly increasing set of measurements on the two transducers, reaching a maximum at the centre of the beam with coordinates shown at "B." Thereafter, as the fish moved away from centre the sequence was reversed on a slightly lower trajectory, the signals falling below threshold as the fish left the beam at point "C." A somewhat similar trajectory has been observed repeatedly on different fish with variations related to behaviour and the precise path of the fish through the acoustic beam. The features of Fig. 2 that distinguish it from data in Fig. 1 for spherical targets are the slight spread of the two arms of the trajectory and the higher average slope $a = 0.79$ ($r = 0.91$) through the array. In observations of fish in various situations, this pattern shows variability in the distance and ratios between points, the direction and degree of separation of the two arms of the trajectory, and the average slope a, calculated from eq. (10). Variations in these parameters are related to position and behaviour of the fish. Comparison between Fig. 1 and 2 illustrates the characteristic difference between a moving fish target and spherical reference targets when the two types of targets are observed under identical experimental conditions.

Figure 3A shows the frequency distribution of eq. (10) slopes, a, for 19 similar sets of observations of 35- to 40-cm cod in the tank. Each slope was based on at least 20 observations per fish as the individual swam slowly across the diameter of the beam. Correlation coefficients of the slopes plotted for each individual fish ranged from 0.91 to 0.98, indicating low spreads between incoming and outgoing trajectories. The average slope, $a = 0.62$, is statistically significantly higher than the 0.5 expected for ideal acoustic targets.

Figure 4 shows trajectories for four fish observed in the course of measurement of the TS of the copper sphere while the ship was moored at the BIO wharf. The copper ball (Fig. 1) was observed at a range of about 11 m. The individual fish appeared at a range between 10 and 14 m. Each fish is represented by few points, indicating a relatively rapid rate of swimming through the beam. The fish were too far from the surface to be seen, but were probably the small "harbour pollock" (*Pollachius virens*) which were caught in the vicinity. The general patterns shown in the figure conform with that illustrated in Fig. 2. The array of slopes derived from over 100 such sets of observations of single fish (Fig. 3B) shows a somewhat higher average slope ($a = 0.95$) and a somewhat wider variation than was found in the experimental tanks.

Data from field surveys yield information on individual fish which is comparable with that shown in Fig. 4. In this situation, with fish at ranges of between 70 and 200 m from the transducer, the maximum number of successive pings on a given fish is a function of depth, the towing speed, and the distance to either side of the centre line of the target's apparent transit through the beam. In calculating the slopes for single targets for this analysis, we have restricted the data to targets that were clearly separated from other targets, that were seen in at least three successive pings, and for which the range of EL measured on the narrow beam spanned at least 5 dB. The results for 144 adult cod detected above bottom at a range of between 70 and 200 m in the Gulf of St. Lawrence during June 1986 are shown in Fig. 3C. The average slope, $a = 0.86$, is intermediate between that found in the tank and that obtained at the BIO dock, but is not statistically different from the average slopes obtained at the dockside. Nor is the array of slopes different from arrays obtained with other species on other occasions and in other survey areas. Our field experience to date suggests an average slope $a = 0.75$ with a standard error of about 0.04 for dual-beam data from fish echoes analyzed in the manner of eq. (10).

It is clear from inspection of the figures that results such as we have obtained can be used for estimates of the average TS of each fish and a variance of the averages. However, a number of options appear to be available for carrying out the analyses. When a single-beam acoustic system is used in fish detection, the one transducer serves as transmitter and receiver. In the ECOLOG system, the narrow beam is used in this manner and the distribution of the results for the narrow beam is represented by projection of the plotted points onto the abcissa of Fig. 1, 2,

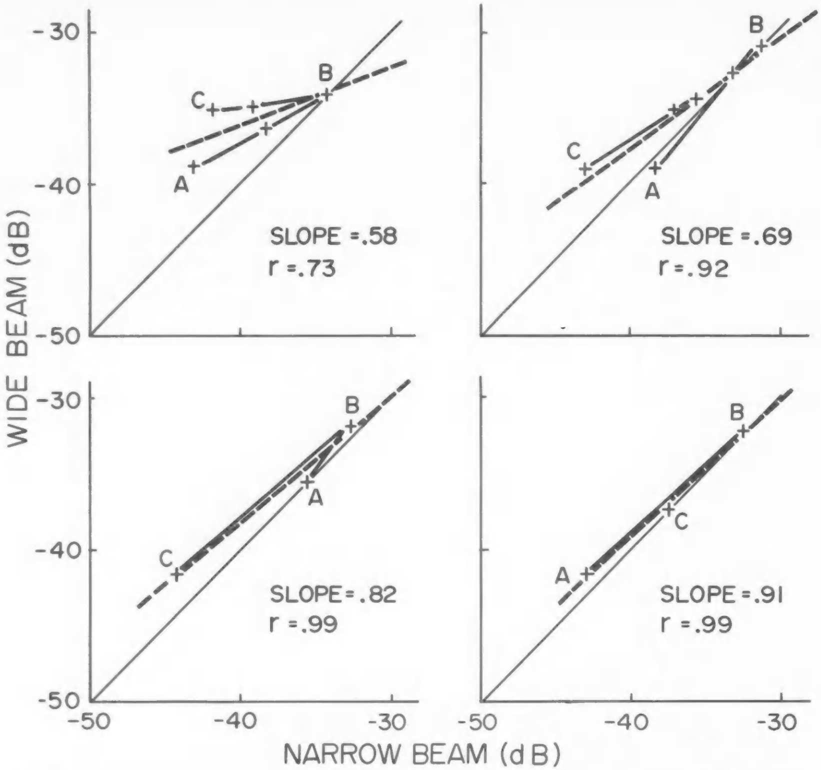

FIG. 4. Trajectory of received echo level of the wide and narrow beams for four separate fish observed during calibration studies at BIO wharf. A, Fish entering beam; B, fish near axial position; C, fish exiting beam. The broken line is the overall linear regression.

or 4. Their standard deviations are shown in Table 1. According to conventional theory, the (2W–N) calculation of target strength, as in eq. (9), would suggest projecting each data point along a line with a slope $a = 0.5$ to an intersection with the 1:1 diagonal. For all of the spherical targets, which had an empirical slope of approximately 0.5, such a (2W–N) calculation shown in Table 1 results in estimates of TS which have standard deviations significantly reduced from the single-beam estimates. In the case of the steel sphere, for example, the standard deviation of the (2W–N) estimates of target strength is reduced by more than one order of magnitude. It is also evident from the figures and Table 1 that the directivity effect of the non-ideal fish targets, resulting in a higher slope for eq. (10), nullifies much of the variance reduction afforded by the dual-beam theory for ideal targets. While on average for spherical targets the eq. (9) algorithm reduces the standard deviation of TS by a factor of more than 5, reductions in standard deviation associated with free-swimming fish are of the order of only 1.5.

These considerations suggest that there should be distinct advantages to using dual-beam data in such a manner that TS is based on projections of the empirically derived slopes rather than on the theoretical slope for ideal targets. The relative effects of doing so are illustrated in Table 1 for the fish illustrated in Fig. 2 and 4. The data show average EL values for narrow and wide beam and an empirically calculated average TS based on the average eq. (10) slope of 0.75 applied to all fish for comparison with on-axis TS calculations based on the theoretical 0.5 slope. The effect of the dual-beam corrections on the variance depends on how closely the average value used approaches the individually observed slopes. In these five fish the 0.75 correction substantially lowers the average standard deviation based on the alternative calculations. In this case, the individual corrected average TS values based on the 0.75 correction have been calculated at a point representing their average distance off axis. In practice, the best value for the slope and the relation of average TS to fish size would be set by a field calibration of "acoustic size" against measured fish samples from the population.

Discussion and Conclusions

The foregoing analysis of acoustic reflections from various regular targets under a variety of conditions indicates that the dual-beam acoustic system works according to theoretical expectations with considerable precision. However, results for living single fish targets moving normally through the acoustic beam give a result that is different from reflection from ideal spherical targets. This is shown with fish in the tank, and with fish detected singly at various ranges in the field, under both laboratory and field conditions. It can only be concluded that the difference between fish and ideal spherical targets must reflect a property of fish as targets, not of the acoustic system as such. This deviation from expectations of the theory of ideal spheres indicates that there is in our results a fish directivity effect which, when the target is moved systematically through the beam, leads to a bias in acoustic measurement of the size of fish targets if estimation is simplistically based on data treatment which does not recognize this effect. Since the apparent size of a target affects the calculated volume in which it is seen, effect on size estimation must also cause a bias in density estimation.

The reasons for the anomalous behaviour of fish echoes seem well enough, if incompletely, known. It is a common finding that a major part of the echo of a fish comes from the gas-filled swimbladder (Clay and Heist 1984). However, the composition of the target by itself does not account for the difference in eq. (10) slope between fish and solid spheres, since with spherical physical targets there was no essential difference between solid metallic spheres and air-filled balloons. We also observed a similarity in slopes regardless of the fact that our solid targets were near perfect spheres, while the balloons were oblong shapes.

Indications of effects of the sort we have found in the field have been implied in a number of laboratory studies, among which that of Foote (1985) provides an especially clear indication. He modelled and measured the relation of the aspect of preserved swim bladders of small pollock to their apparent TS using acoustic frequencies and wavelengths similar to ours. His results showed significant directivity effects, evidenced by a decrease in measured echo levels for small aspect angles; the effect was slightly asymmetrical from the head and tail ends of the fish. Since fish moving through the beam of the transducer show changes in aspect of some 6–8° as a result of the perspective from which they are seen, Foote's directivity effects may well account for both the higher slopes and the asymmetrical trajectories we have observed. The magnitude of the effect might well be described by the introduction of an appropriate fish directivity pattern in eq. (1). A simple model of perspective effect which we constructed on the basis of Foote's data gave a Fig. 1 type slope, $a = 0.70$, rather than the 0.5 expected from a sphere.

Two additional phenomena may influence our results. First, Traynor and Ehrenberg (1979) noted that noise in acoustic systems will lead to a bias in estimates of average TS derived from the ratio of signals from the two transducers. They demonstrated the effects in a simple simulation model which formed the basis for editing their data on fish size. While such effects appear to be small relative to those displayed in our data, any such influence will indeed increase the apparent slope of eq. (10) and needs to be taken into account. Second, Huang and Clay (1980) have examined fish echoes in some detail, using a simple imaging device based on a single-beam system. They concluded that distortions in the frequency distribution of TS measurements result from the complex tissue composition and shape of the target and from its mobility, implying a more complex theory of fish reflection than the usual one-parameter Raleigh (Clay and Heist 1984). Their theories would also imply a higher variability and possibly an upward bias in the estimate of slope from dual-beam data. Neither of these theories appears to account satisfactorily for the pattern of regularity in the trajectories shown in Fig. 2 and 4, or for the difference in the angle between them under certain conditions.

Regardless of the exact explanation of the observed patterns, their presence will have significant consequences for the acoustic estimation of various parameters of fish size and abundance. In particular, it would appear that where fish directivity is present but not accounted for, it introduces as much variance into measurement of dual-beam TS as was present in the single-beam systems it was intended to improve on. This increased variance will enhance the type of bias in estimated average TS to which Traynor and Ehrenberg (1979) originally drew attention. An error in estimation of average size also affects the estimate of volume in which a target is seen. Such effects therefore also mean that there will be comparable errors in estimates of volume sampled, and hence in density or biomass estimates based on them.

Where acoustic systems can be used to give repeated measurements on single fish, relations of the sort described by eq. (10) and illustrated in Fig. 2 and 4 should make it possible to correct for this bias empirically. The results would appear to enable one to determine the necessary parameters of fish size and abundance with useful precision.

Acknowledgements

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Otolith Microstructure of Larval Herring (*Clupea harengus*): Image or Reality?

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When assessed with light microscopy, daily increment formation did not appear to occur in the otoliths of known-age larval herring (*Clupea harengus*). Increment counts underestimated age in all larvae. The age–increment discrepancy increased curvilinearly with age and appeared to stabilize after 50–60 d. Both the magnitude and rate of increase of the discrepancy were consistent with a hypothesis of resolution-limited increment visibility; models of daily otolith growth indicated that discrete daily growth increments would not be resolvable with a light microscope for the first 15–20 d after hatch. The hypothesis was also consistent with the observed effects of otolith polishing, a systematic difference in increment counts between different-sized sagittae in the same larvae, and other published reports of apparent nondaily increment formation in slow-growing pelagic larvae. Previous reports of growth rate limited increment formation appear to provide an empirical description of the same phenomenon. Otolith-based age, growth, and mortality estimates can be expected to be biased if resolution effects are ignored. However, various procedures are available for the identification of potentially sensitive species and samples.

La lecture au microscope optique des otolithes de larves de hareng (*Clupea harengus*) d'âge connu n'a pas permis de mettre en évidence la formation de stries journalières d'accroissement. L'âge de toutes les larves a été sous-estimé. L'écart entre l'âge réel et l'âge estimé augmentait de façon curviligne avec l'âge et semblait se stabiliser après 50 à 60 j. Tant l'ampleur que le taux d'accroissement de l'écart satisfont à une hypothèse voulant que la visibilité des stries d'accroissement soit limitée par la résolution; des modèles de la croissance journalière des otolithes ont indiqué que les accroissements journaliers ne pourraient être mis en évidence par un microscope optique pour les 15 à 20 premiers jours suivant l'éclosion. Cette hypothèse se défend également si on considère les effets observés du polissage des otolithes, les différences systématiques des lectures des sagittas de taille différente provenant des mêmes larves et les rapports faisant état d'une formation apparemment non journalière des stries d'accroissement chez des larves pélagiques à croissance lente. D'autres rapports indiquant que la formation des stries d'accroissement serait limitée par la vitesse de croissance semblent apporter une description empirique du même phénomène. On peut penser que les estimations de l'âge, de la croissance et de la mortalité reposant sur la lecture des otolithes sont biaisées lorsqu'il n'est pas tenu compte des limites de résolution. Ceci dit, il existe diverses méthodes permettant de reconnaître les espèces et échantillons que ce phénomène pourrait toucher.

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In 1982, Geffen challenged the concept that daily growth increments in the otoliths of young fishes provide an accurate index of age. Data collected from known-age larval herring (*Clupea harengus*) suggested that the frequency of increment formation was less than daily in larvae with a suboptimal growth rate. Further, she reported that the rate of increment formation was a curvilinear function of somatic growth rate below some threshold limit. Nondaily increment formation has since been corroborated in larval herring (Lough et al. 1982; McGurk 1984), as well as a variety of other fish species with slow-growing larval phases (Methot and Kramer 1979; Laroche et al. 1982; Bergstad 1984; Campana 1984). Such reports are of particular concern to those who have assumed a daily rate of increment formation in their age-structured analyses (Townsend and Graham 1981; Laroche et al. 1982; Graham et al. 1984; Penney and Evans 1985). There is no reason to doubt the apparent universality of daily increment formation in young fishes under adequate growth conditions (Campana and Neilson 1985). However, the number of reported exceptions, all of which were from temperate pelagic larvae, suggests the existence of some other mechanism which could conceivably complicate or invalidate age interpretations derived from otolith microstructure examination.

Several hypotheses can be invoked to explain apparent increment formation rates of less than one per day. The growth rate limitation hypothesis of Geffen (1982) has been cited as consistent with the results of a number of studies (Bergstad 1984; McGurk 1984). An alternative hypothesis, that of limited observer resolution, was first presented when it was noted that narrow daily increments could only be seen after adequate otolith preparation (Campana 1984). An expansion of this hypothesis suggested that it was theoretically possible for daily increments to form at or below the resolution limit of even a "perfect" light microscope (Campana and Neilson 1985). Such a situation would result in an apparent increment formation rate that was lower than the true rate. Of course, the resolution and growth rate hypotheses are not mutually exclusive. It is the objective of this paper to demonstrate that novel mechanisms of growth-limited increment formation need not be invoked to explain instances of apparent nondaily increment formation in slow-growing pelagic larvae. Through use of data collected from laboratory-reared larval herring, we will show that an apparently nondaily increment formation rate is to be expected in the otoliths of many pelagic larvae. Realistic light microscopic resolution limits will then be used to reconcile observed and expected increment counts both in this study and elsewhere. Finally, we will assess the implications of our findings with respect to applied otolith microstructure analyses.

Materials and Methods

Herring larvae were reared in the laboratory from fertilization to at least the time of metamorphosis. Fertilized eggs were obtained from hand-stripped adults collected in the Iles Verte region of the St. Lawrence Estuary in June 1985. Larvae were kept in 50-L recirculating, closed-system tanks and fed a continuous supply of rotifers, *Artemia* and/or wild zooplankton; rearing details are available elsewhere (J. Munro and J. A. Gagné, unpubl.) Lighting was fluorescent on a 16 h : 8 h day–night cycle, while temperature was kept roughly constant at 10–12°C. Diel temperature fluctuations, if present, were less than 0.25°C/d. Survival was on the order of 97.8%/d.

Larvae were sampled on a daily basis for the first 35 d of the experiment and at 2- to 8-d intervals thereafter. After having recorded total length to the nearest 0.1 mm ($N = 194$), all lapillar and sagittal otoliths were removed from a random subsample of the larvae ($N = 65$), cleared of adhering tissue, and mounted on standard microscope slides with Krazy Glue. Maximum otolith diameter was measured to the nearest micrometre with an ocular micrometer.

Microscopic Examination

Microstructural examinations were made at 1250× with a research quality microscope fitted with planachromat objectives. Theoretical resolution of the system was calculated with the following equation:

$$R = \frac{\lambda}{2\text{NA}}$$

where R is the smallest visible distance between two structures, λ is the wavelength of light used, and NA is the overall numerical aperture of the system. Numerical apertures for both the objective and condenser lenses met or exceeded 1.25; however, since only the objective lens was immersed in oil, the NA for the condenser (and hence the whole system) was limited to 0.90–0.95. Accordingly, the theoretical resolution limit of the system under blue light was approximately 0.24 μm. This value is subject to some uncertainty, since several formulae for resolution limits are available (Eastman Kodak Co. 1980). However, functional limits are invariably higher than those derived theoretically and are subject to influence from the visual acuity and microscopic technique of the observer.

Increment counts were made of all sagittae and replicated at least twice by the same reader. Age was unknown to the reader at the time of otolith examination. Lapillar microstructure was difficult to interpret in larger larvae and was not examined further. Counts were made along the longest clear axis between the otolith periphery and a well-defined medial increment hereafter referred to as a "hatch check." While the nature of the hatch check could not be determined with certainty, its diameter (mean ± 95% C.I. = 23.0 ± 0.38 μm) in relation to the size of newly hatched larval sagittae indicated that it was formed at or within several days of hatch. Therefore, the check was used as a clearly defined temporal benchmark from which counts could be initiated. Subdaily increments apparent in many of the older otoliths were treated as such and not counted. While the distinction between daily and subdaily increments can seldom be made with total objectivity (Campana and Neilson 1985), any misinterpretation of subdaily increments as daily increments would serve to strengthen the arguments which are to follow.

Otolith microstructure was examined in specimens both before and after polishing. Increment counts were made of all otoliths prior to polishing and of all those greater than 30 μm in diameter after polishing. The latter were prepared with a graded series of aluminum oxide lapping films (30–3 μm grit size) to a plane just above the midplane. Hatch check diameters of polished specimens were measured to the nearest micrometre; post-hatch growth radii were calculated as one half the difference between the otolith and hatch check diameters. Twelve sagittae prepared for scanning electron microscopy (SEM) were given a final polish to the midplane with a 0.3-μm grit film, etched for 2–4 min in 0.1 *M* ethylenedinitrilotetraacetic acid (EDTA), and coated with gold–palladium.

Although etching was satisfactory in the region of the otolith formed after 30 d, it was poor in the area of interest (the perinuclear area). If the region of inadequate etching had been restricted to the area where growth increments were poorly resolved with a light microscope, the argument could be raised that no increments were present to be etched. However, some of the increments that were clearly visible with light microscopy remained unetched after EDTA treatment. It is possible that other etching treatments would have produced superior preparations. However, the SEM results available to us could be used neither to support nor reject hypotheses of daily increment formation, and SEM preparations were not continued.

Data Analysis

Daily otolith growth was modelled with both linear and nonlinear regressions. All regression parameters were estimated by least-squares methods. Data input consisted of one otolith observation per larva (selected at random from the two available), except in the resolution model where both sagittae were used. Residuals from the models were given careful examination, particularly near the origin. Models were accepted only in the absence of patterns in the residuals. The significance level was set at 0.05 for all tests.

Results

Increment Formation Frequency and Age

The two pairs of otoliths present at hatch, the sagittae and lapillae, were initially similar in both size and shape. While Lough et al. (1982) referred to the anterior pair as asteriscii, their position and subsequent development indicate that they were indeed lapillae. Sagittal growth in young larvae was exponential relative to larval growth. As a result, increment width tended to increase with age, at least until age 50 d; however, increments were narrow and difficult to see in larvae less than 20 d old.

In virtually all studies where the frequency of increment formation has been determined, a simple linear regression has been fitted to age and increment count data. As a first step, the same approach was adopted here. The resultant slope of 0.78 was both significant and highly correlated ($r^2 = 0.93$), suggesting that increments did not always form on a daily basis. However, the residuals of the linear model were markedly curvilinear at young ages, indicating that a simple linear model is inappropriate for these data (Fig. 1).

As a working hypothesis, increments were assumed to have formed daily during the experiment. Departure from such a model is presented in Fig. 2. The discrepancy between age and increment count increased rapidly with age; in the most extreme case, increment count underestimated larval age by 72%. There was no significant increase in the discrepancy after 30 d; stabilization occurred at a mean level of 17. This age-structured pattern in the discrepancy suggested a daily frequency of increment formation in older fish, and a lower formation rate in the younger fish. Daily increment formation in older fish was substantiated through an age–increment regression slope not significantly different from 1.0 in fish greater than 30 d old.

Increment Formation Frequency and Growth Rate

Larval growth rate varied nonlinearly with age. An average growth rate of 0.29 mm/d was maintained in each tank through day 80, although there were indications of growth cessations at irregular intervals. Mean growth rate before day 40 was significantly lower (0.24 mm/d) than that between days 40 and 80 (0.36 mm/d), while that in the first 9 d was not (0.30 mm/d). Therefore, age-related trends in growth rate may have been present, but were inconsistent through time.

Otolith growth rate was a better predictor of increment formation frequency than was larval growth rate ($P < 0.05$). Otolith size was significantly correlated with larval size. However, in an intralarval comparison of both sagittae from larvae less than 40 d old, increment counts were significantly higher in the larger of the two otoliths (paired *t*-test, $P < 0.001$). Further, the difference in increment number between the two otoliths was significantly related to the size difference between the two (Fig. 3). Therefore, previous reports of a correlation between larval growth and increment formation frequency (Geffen 1982; McGurk 1984) are more correctly described as a correlation between otolith growth rate and increment formation frequency.

Resolving Power

The influence of reduced resolving power on counting accuracy was demonstrated in a comparison of otoliths before and after polishing. Increment counts in the former were not significantly greater than those in the latter in sagittae with diameters of less than 35–40 μm. Polishing significantly increased both counts and clarity in larger otoliths, and was clearly mandatory when diameters reached or exceeded 50 μm. The age–increment discrepancy (described above) was documented in polished otoliths; however, its magnitude would have been considerably greater in the absence of polishing. Increments obscured by overlying material in the larger otoliths were those nearest the hatch check and were difficult to see under even ideal conditions. However, three rather broad (approximately 1 μm), clearly visible "increments" were visible around the hatch check in most otoliths from larvae greater than or equal to 15 d old. While these "increments" were most apparent in unpolished preparations, they could also be seen after polishing, particularly under conditions of poor focus. Under closer examination, each broad increment could be decomposed into a larger number of narrow increments; the widths of the latter were consistent with those of adjacent increments.

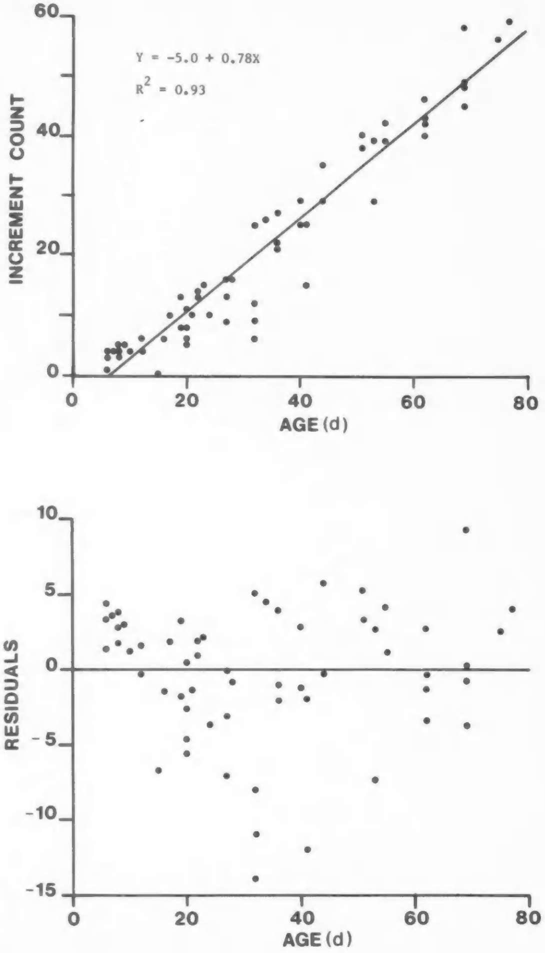

FIG. 1. Increment count as a function of age in laboratory-reared larval herring. The pattern in the residuals indicates that the simple linear regression that has been fitted is inappropriate.

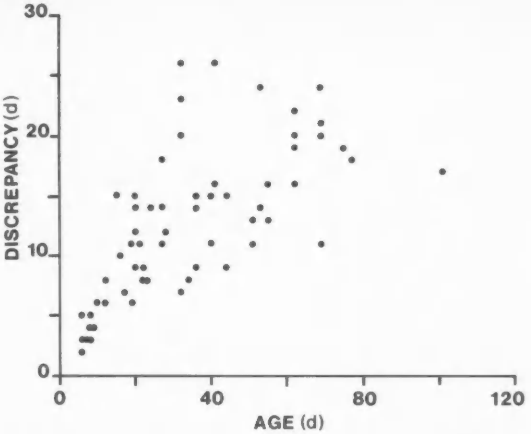

FIG. 2. Discrepancy between age and increment count (age minus count) as a function of age in laboratory-reared larval herring.

Theoretical and Observed Daily Increment Widths

The resolution hypothesis states that larval herring increments formed in the first weeks after hatch will be too narrow to resolve with a light microscope. A direct test of this hypothesis with SEM was inconclusive (see Materials and Methods). Accordingly, the daily growth of the otolith was modelled with the following logistic equation:

$$(1)\quad Y_a = 22.83 + \frac{357.3}{1 + \exp(-0.09647(\text{age} - 60.08))}$$

where Y_a = otolith diameter (micrometres) at age a (Fig. 4). The model was highly significant (Table 1), with the distribution of the residuals indicating that the model fit the data well, including near the origin. Assuming daily increment formation from the date of hatch, theoretical (or expected) increment width at age a (Theor_a) was calculated as

$$(2)\quad \text{Theor}_a = (Y_a - Y_{a-1})/2.$$

This assumes symmetric growth on either side of the nucleus along the measurement axis. Visual assessment of otolith shape to day 100 indicated that this was a realistic assumption. Theoretical increment widths increased exponentially from the time of hatch to age 40–50 d; they peaked at age 60 d and declined thereafter (Fig. 5). Given daily increment formation in the herring otoliths, the first 15 increment widths were predicted to be below the limit of resolution of our microscope system. Note that this value is similar to the observed age–increment discrepancy in older (>25 d) larvae (Fig. 2). Even with a "perfect" light microscope (with a resolution limit of 0.16 μm under blue light), it would have been impossible to see the first 11 daily increments.

Apparent increment widths (Appar) were estimated in a manner similar to that of Theor. The logistic equation

$$(3)\quad Y_c = 26.15 + \frac{318.3}{1 + \exp(-0.1341(\text{count} - 41.04))},$$

where Y_c = otolith diameter (micrometres) at increment count c, fit most of the data well (Table 1; Fig. 6). Nonrandomly distributed residuals were noted in otoliths with a diameter of less than 29 μm, but not in larger otoliths. Apparent increment width increased with the first 41 counts and declined thereafter (Fig. 7): the general pattern was similar to that observed in the theoretical width model although there was a lag consistent with the age–increment discrepancy. The first seven apparent widths were narrower than the limit of resolution of our microscope (0.24 μm) but considerably larger than those predicted by the theoretical model.

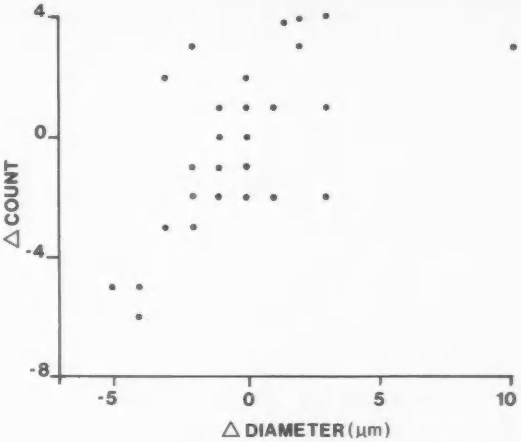

FIG. 3. Intralarval variation in increment count as a function of intralarval variation in otolith diameter. Intralarval variation was assessed as the difference between the two sagittae of a given larva. The relationship is plotted for those larvae ≤40 d old.

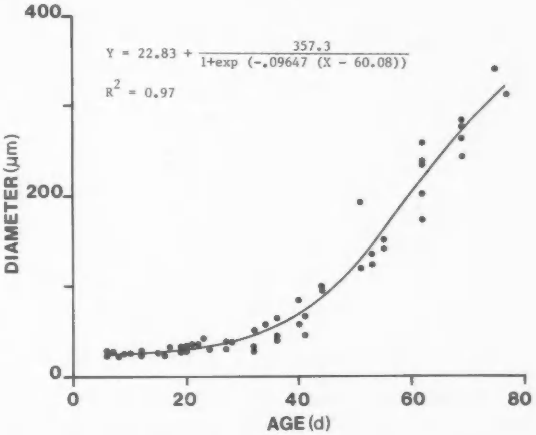

FIG. 4. Relationship between otolith diameter and post-hatch age as fitted with the logistic equation. Model results are presented in Table 1.

Resolution Model

If inadequate resolution was responsible for the observed discrepancy between age and increment count, it should be possible to reconcile observed and expected counts in a model which assumes that daily increments are visible as distinct structures only if their width exceeds a specified resolution limit. Narrower increments would appear distinct only if pooled with adjacent increments in sufficient number to exceed the resolution limit.

Equation 1 was used to determine the mean pattern of

TABLE 1. Parameter estimates, associated error terms, and ANOVA's for the logistic growth models fitted in Fig. 3 and 5. Theoretical width model: otolith diameter (μm) vs. age (d); apparent width model: otolith diameter (μm) vs. increment count; $Y = a + b(1 + \exp(-c(x - d)))^{-1}$.

Model	Source of error	Sum of squares	df	Mean square	R^2	Variable	Coefficient	SE
Theor	Model	944 105	4	236 026	0.971	a	22.83	3.94
	Error	14 453	60	241		b	357.3	34.8
						c	0.09647	0.01085
						d	60.08	2.18
Appar	Model	940 707	4	235 177	0.964	a	26.15	3.80
	Error	17 851	60	298		b	318.3	21.4
						c	0.1341	0.01519
						d	41.04	1.133

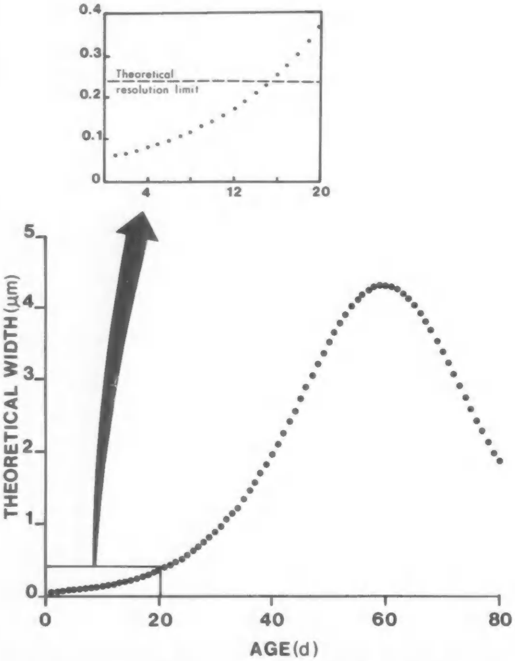

FIG. 5. Theoretical (or expected) daily increment width as a function of age in larval herring. Theoretical values were derived from a fitted otolith growth model (Fig. 4; Table 1).

expected daily increment widths in our sample of otoliths (Fig. 5). To adjust this pattern to the size of an individual otolith, a scaling factor (SF) was used to adjust the sum of expected increment widths to the observed otolith post-hatch growth radius (PHGR). For otolith i of age a:

$$\mathrm{SF}_i = \mathrm{PHGR}_i / \sum_{1}^{A} \mathrm{Theor}_a$$

where Theor is the theoretical width of the ath daily increment. The model is discrete in that increments narrower than the functional resolution limit (FR) were pooled in integer combinations only.

Mathematically, increment count

$$C = \sum_{1}^{a} C_a$$

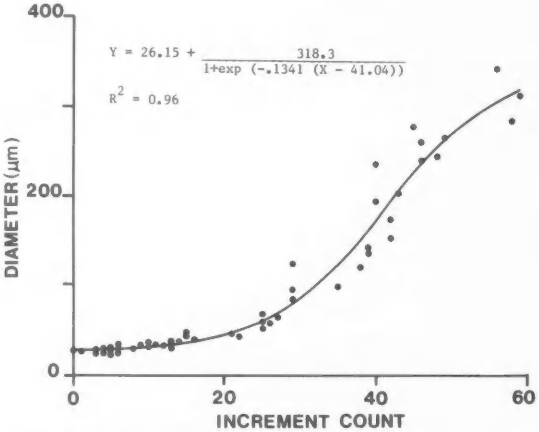

FIG. 6. Relationship between otolith diameter and increment count as fitted with the logistic equation. Model results are presented in Table 1.

for each daily increment in a given otolith, where C_a is calculated according to the following algorithm:

(1) $a = 1$
(2) $n = 0$
(3) If $\left(\mathrm{SF} \times \left(\sum_{j=a}^{a+n} \mathrm{Theor}_j\right) \geq \mathrm{FR}\right)$, go to 6
(4) $n = n + 1$
(5) Go to 3
(6) $C_a = 1$
(7) $a = a + n + 1$
(8) Go to 2.

The fit of the model was assessed through comparison of predicted and observed increment counts. Fit was maximized through iterations where the magnitude and constancy of functional resolution were varied. Maximization criteria consisted of a 1:1 relationship between observed and predicted increment counts, a high correlation coefficient, and a randomized residual pattern. While high correlation coefficients could be achieved when resolution remained invariant with otolith diameter ($R \geq 0.94$), it was not possible to produce a randomized residual pattern and a slope of 1.0 at a constant resolution limit. Models that incorporated linear increases in resolution with otolith diameter produced an improved, but still biased, fit. Fit was maximized and bias minimized when resolution increased with the square root of otolith diameter

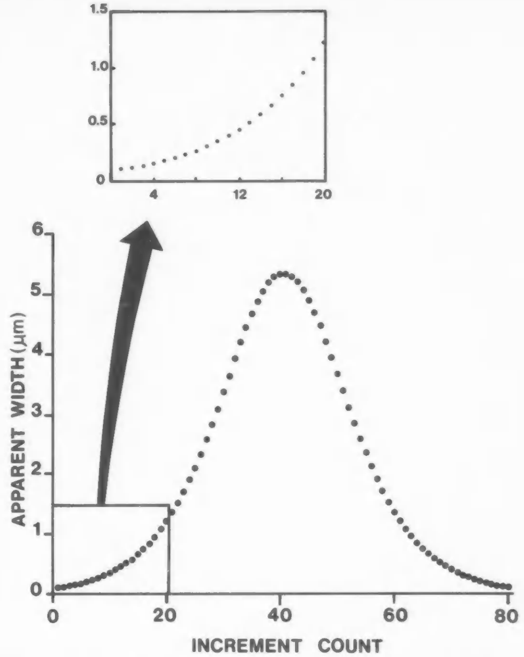

FIG. 7. Apparent increment width as a function of increment count in larval herring. Apparent values were derived from a fitted otolith growth model (Fig. 6; Table 1).

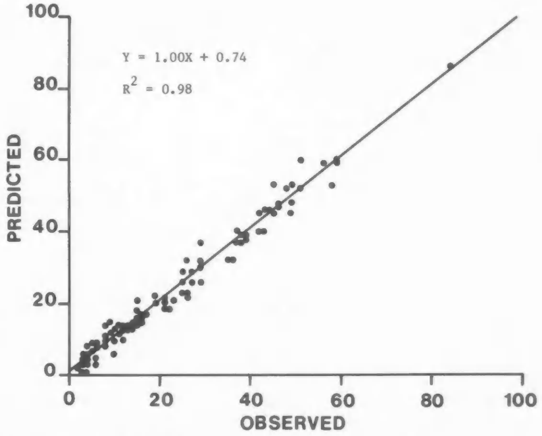

FIG. 8. Relationship between observed and predicted increment counts resulting from a model incorporating resolution-limited visibility of increments. See text for details of model.

($\mathrm{FR} = 0.16 + 0.02\sqrt{\mathrm{Diam}}$). The resultant relationship between observed and predicted increment counts had a slope of 1.0, was highly correlated, and was linear (Fig. 8). The model's assumption of a square root relationship between otolith diameter and resolution was justified on the basis of empirical observations of the otolith thickness to diameter ratio and the fact that light transmission is affected by passage through the lower (unpolished) half of the otolith. Functional resolution, as derived from the model, was 0.25 μm for the smallest otolith, increasing to 0.71 μm for the largest specimen. The resolution estimate for the smallest otolith was nearly identical to the theoretical calculation for our microscope system (0.24 μm), thus providing an independent test of the validity of the model and its assumptions.

Discussion

A daily rate of increment formation was not evident in known-age larval herring otoliths when assessed with light microscopy. Simple linear regression estimates of the apparent rate of increment formation (0.78) were consistent with other studies of larval herring with similar growth rates (Geffen 1982: Lough et al. 1982; McGurk 1984). However, the distribution of the residuals indicated that the simple linear model was inappropriate for these data; the apparent increment formation rate was much lower in young larvae than in old. Daily increments were almost certainly formed in larvae older than 30 d. Given the discrepancy between age and increment count in the younger larvae, the question of the most plausible mechanism arises: are daily increments forming with widths below the resolution limit of light microscopy, or are increments forming at irregular, nondaily intervals?

Resolution Hypothesis

The resolution hypothesis provides a causal and empirical explanation of apparent microstructural anomalies through a biologically and physically plausible mechanism. The hypothesis states that daily increments with widths below the functional resolution limit of a light microscope can and do form in some larval otoliths. Since these increments would not be visible as distinct structures, an apparent increment formation rate of less than 1 would result. The strength of the hypothesis lies in its precise mathematical prediction of age-count anomalies and its attendent explanations of apparently unrelated phenomena. It is fully consistent with the results of this and other studies and does not invoke any novel mechanisms of otolith growth.

There is no doubt that resolution plays a role in the interpretation of herring otolith microstructure. The comparison of polished and unpolished otoliths provided the most visible evidence of this: similar results have been reported from larval starry flounder (*Platichthys stellatus*) otoliths (Campana 1984). In situations such as these, where some increments were clearly visible without polishing, increment counts could underestimate increment number as a result of omitting the polishing stage.

Can daily increments form with widths that are unresolvable by light microscopy? The results of the otolith daily growth model indicated that daily otolith growth in the first 2 wk after hatch was insufficient to be resolved as discrete daily growth increments with our light microscope. Given the fit of the model and the magnitude of the predicted values, this conclusion is unambiguous even if increment formation started 4.5–6 d after hatch, as has been inferred elsewhere (Geffen 1982; Lough et al. 1982: McGurk 1984). Support for this conclusion comes from the microstructural examination of chinook salmon (*Oncorhynchus tshawytscha*) otoliths, where SEM was used to detect narrow daily increments formed at 5°C (Neilson and Geen 1982), while increment formation had apparently stopped at this temperature when assessed by light microscopy (Marshall and Parker 1982). Similar conclusions were reached in an examination of striped bass (*Morone saxatilis*) otoliths by both SEM and light microscopy (Jones and Brothers 1987). In the latter instance, light microscopic increment counts seriously underestimated the number visible under SEM. Therefore, the conclusion that narrow daily increments were formed in the otoliths of the larval herring at or shortly after the time of hatch is consistent with the resolution problems reported by other workers.

Observed increment counts were closely predicted by the resolution model when realistic functional resolution limits were used. The age–increment discrepancy was predicted to increase quickly to 15–20, increasing only slightly thereafter. This prediction was similar to the observed discrepancy, both here and in several other herring studies where growth rates were similar to ours (Geffen 1982; Lough et al. 1982; McGurk 1984). Since compression of a daily increment sequence would be expected to increase as larval growth rate slowed, the resulting decline in apparent increment formation rate would be fully consistent with the results reported elsewhere (Geffen 1982: McGurk 1984). The model also helped explain the observation of three broad perinuclear "increments" in the first 20 d after hatch (Lough et al. 1982; this study) as aggregates of poorly resolved increments. Such an explanation is intuitively appealing in that it avoids the need for novel mechanisms of otolith growth. A third prediction of the model was met in the observed correlation of differential otolith size and differential increment count between the two otoliths of a given larva. Finally, the model correctly predicted a small increase in the accumulated age–increment discrepancy in older larvae in which daily increments were clearly forming. All of the above suggests that the resolution model accounts for all, not just most, of the age–increment anomalies observed in larval herring. Indeed, given the growth-dependent apparent increment formation rate predicted by the resolution hypothesis, we conclude that Geffen's growth rate limitation hypothesis provides an excellent empirical description of the same phenomenon which we describe here. Therefore, we conclude that Geffen's (1982) observations actually support the resolution hypothesis, with the latter providing the theoretical basis for her observations. It should also be noted that an explicit conclusion of the resolution model is that increment formation occurs daily and independently of growth rate and age. Thus, the model is fully consistent with current concepts of increment formation in fish otoliths (Campana and Neilson 1985).

The lower limit of resolution suggested by the resolution model was very similar to that calculated independently for our microscope system. However, the first-formed increment widths predicted by the apparent width model were significantly smaller. Somewhat counterintuitively, this discrepancy is also consistent with the resolution hypothesis. According to the hypothesis, unresolved increments should aggregate in a number sufficient to be resolvable as a unit. Each of these aggregates would be expected to be of similar width. However, the logistic equation used in the Appar model makes no allowance for a series of units with constant widths near the origin, and this is reflected in the residual pattern near the origin. Thus, all three estimates of resolution were consistent with each other.

Implications

The implications of the resolution model extend well beyond interpretive bias in the otolith microstructure of herring larvae. There are a number of larvae for which increment formation rates of less than one have been reported: examples include anchovy (*Engraulis mordax*) (Methot and Kramer 1979), English sole (*Parophrys vetulus*) (Laroche *et al.* 1982), starry flounder (Campana 1984), and sand lance (*Ammodytes americanus*) (S. W. Richards, Little Harbor Laboratory, 69 Andrews Road, Guilford, CT 06437, pers. comm.). All of these species are similar to herring in that they possess a pelagic larval phase with the potential for minimal otolith growth; where increment widths were reported, the lower limit extended well below 1 μm. In other words, these species are expected candidates for resolution-limited increment counts. Presumably there are many others in which resolution limitations may be expected to occur, particularly in colder waters where growth can be slow. Since light microscopy is the most frequently used and convenient of the otolith examination procedures (Campana and Neilson 1985), resolution limitations may already have introduced error and bias into some published estimates of larval age, growth, and mortality. Of course, the magnitude of those errors would be a function of reported age; estimates for young larvae would be more seriously affected than those for older individuals. And given the prediction that age–increment discrepancies would be expected to vary with growth (Geffen 1982; McGurk 1984), empirical corrections for an apparent age--increment discrepancy (Lough et al. 1982) would be unlikely to be accurate in the absence of independent knowledge of the growth rate through the relevant period.

While not so serious, the age at hatch check formation may also be open to error in resolution-limited otoliths. As first suggested by Lough et al. (1982), an apparently discrete hatch check may actually be composed of an aggregate of unresolved daily increments. This hypothesis cannot be tested with light microscopy alone.

The apparent susceptibility of temperate pelagic larvae to resolution effects is largely a result of their low otolith growth rate relative to that of juveniles. This may account for the few reported examination problems in juveniles. The allometric otolith to body length relationship in larval herring makes the early larval stage particularly sensitive; given such a relationship, daily increment width should increase with age despite constancy in the larval growth rate (i.e Fig, 5), implying that the most serious potential resolution difficulties should occur nearest the nucleus. Similar increases in increment width with age would be expected of other species with an allometric growth relationship. Despite speculation to the contrary (Victor 1986), this form of otolith growth is characteristic of a number of species with pelagic larval phases (i.e. haemulids, Brothers and McFarland 1981; clupeids, Lough et al. 1982; engraulids, Tsuji and Aoyama 1984; gadids, Nishimura and Yamada 1984; pleuronectids, Campana 1984).

Guidelines

Identification of resolution-limited microstructural preparations should be possible in many cases. Direct measurements of increment width, or widths estimated from an observed increment width model such as that described previously (Appar), should provide some indication of the need for further examination. In theory, the calculated resolution limit of the microscope could be used to assess the reliability of the observed increment widths. In practice, however, the former will probably underestimate the true limit due to the influence of sample thickness on resolving power. As a general guideline, we suggest that otoliths where increment widths are less than 1 μm be examined further. Otoliths where increment widths appear to increase in proximity to the nucleus may also be suspect. Polishing will improve resolution in larger otoliths, but will not necessarily identify resolution-limited increments in small otoliths. A more powerful technique is the comparison of increment counts between left and right otoliths; where counts are greater in the larger of the two otoliths, resolution limitations should be suspected. Of course, such a comparison is only appropriate for the growth zone where increment widths are narrow. Note that this technique is suitable for identifying a resolution problem, but cannot be used to assess the magnitude of the age–increment discrepancy. Indeed, none of the identification measures listed above appears to be suitable for estimating the magnitude of the discrepancy. Where necessary, it may be possible to estimate approximate discrepancies through knowledge of the otolith – larval length relationship, the observed increment width pattern, larval growth rate, and other biological characteristics.

Several procedures are available to maximize resolution during light microscopic examination of otoliths. Polishing and thin sections derived from two-sided polishing can produce superior preparations (Campana and Neilson 1985), thus improving resolution. Resolution can also be improved through careful attention to the effective numerical aperture of the microscope, which can be more important than increased magnification (Eastman Kodak Co. 1980). Substantial increases in both magnification and resolution require application of SEM, a technique that has already been applied successfully to increments unresolvable by light microscopy (Jones and Brothers 1987). However, SEM will not always be appropriate for otolith examination, as was noted in this study. Etching for SEM requires the presence of differences in chemical composition between incremental and discontinuous zones within a daily increment. These differences also render the increment visible with light microscopy as a distinct bipartite structure. Given the low visual contrast characteristic of some larval increments, it may be that SEM will prove no more useful in their examination than will resolution-limited light microscopy.

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Effects of Mammalian and Salmon Gonadotropin-Releasing Hormones and Analogues on Plasma Gonadotropin Levels and Ovulation in Coho Salmon (*Oncorhynchus kisutch*)

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Van Der Kraak, G., E. M. Donaldson, H. M. Dye, G. A. Hunter, J. E. Rivier, and W. W. Vale. 1987. Effects of mammalian and salmon gonadotropin-releasing hormones and analogues on plasma gonadotropin levels and ovulation in coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci. 44: 1930–1935.

The effects of intraperitoneal injections of mammalian gonadotropin-releasing hormone (mGnRH) and salmon gonadotropin-releasing hormone (sGnRH; [Trp^7,Leu^8]-mGnRH) as well as analogues of each peptide on plasma gonadotropin levels and ovulation in coho salmon (*Oncorhynchus kisutch*) were investigated. The native peptides had similar potencies in terms of the magnitude and duration of the gonadotropin release response. Analogues including the D-Ala^6 and (imb$_z$l) D-His^6 derivatives of [Pro^9-NEt]-mGnRH and the D-Arg^6 and D-Ala^6 derivatives of [Pro^9-NEt]-sGnRH stimulate a more prolonged increase in plasma gonadotropin levels than native forms of these peptides. Each of the analogue peptides at a dosage of 0.2 mg/kg body wt induced a high rate of ovulation; the native salmon peptide at the same dosage was also effective although the time of ovulation was delayed compared with the response to analogue peptides. A higher dosage of the native salmon peptide (1.0 mg/kg body wt) was less effective in stimulating gonadotropin release and failed to induce a high rate of ovulation. Our studies demonstrate that several analogues of mGnRH and sGnRH have superactive agonist activity in coho salmon and are effective in inducing ovulation in this species.

On a étudié les effets d'injections intrapéritonéales de la gonadolibérine de mammifère (mGnRH), de celle de saumon (sGnRH; [Trp^7, Leu^8]-mGnRH) ainsi que d'analogues des deux peptides sur l'ovulation et les concentrations de la gonadotrophine dans le plasma chez le saumon coho (*Oncorhynchus kisutch*). Les peptides natifs étaient similaires en ce qui concerne l'ampleur et la durée de la réponse de libération de la gonadotrophine. Les analogues, comprenant les dérivés à D-Ala^6 et à (imb$_z$l) D-His^6 de la [Pro^9-NEt]-mGnRH et à D-Arg^6 et à D-Ala^6 de la [Pro^9 -NEt]-sGnRH ont stimulé une augmentation de plus longue durée des concentrations de la gonadotrophine plasmatique que les formes natives de ces peptides. À la dose de 0,2 mg/kg (poids corporel), tous les peptides analogues ont entraîné un taux élevé d'ovulation; à la même dose, la forme native du peptide du saumon a également été efficace, mais l'ovulation a été retardée par comparaison à la réponse obtenue avec les formes analogues. Une dose plus élevée de la forme native du peptide du saumon (1,0 mg/kg [poids corporel]) a stimulé moins efficacement la libération de la gonadotrophine et n'a pas donné un taux élevé d'ovulation. Nos études démontrent que plusieurs analogues de la mGnRH et de la sGnRH ont une action agoniste très élevée chez le saumon coho et assurent une induction efficace de l'ovulation chez celui-ci.

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In recent years, there has been an increased effort to utilize gonadotropin-releasing hormone (GnRH) in place of pituitary extracts to induce ovulation in teleosts (see Crim et al. 1986). Much of this work has involved use of the [D-Ala^6,Pro^9-NEt] derivative of mammalian gonadotropin-releasing hormone (mGnRH) which stimulates a more prolonged increase in circulating gonadotropin levels than mGnRH (Peter 1980, 1986; Van Der Kraak et al. 1983). On the basis of recent studies, it is known that there are several different molecular forms of GnRH throughout vertebrates (Sherwood 1986). For example, the major form of GnRH isolated from chum salmon (*Oncorhynchus keta*) hypothaami (sGnRH) differs from the structure of mGnRH by the substitution of tryptophan and leucine in positions 7 and 8, respectively (Sherwood et al. 1983). Further studies have shown that sGnRH is present in a wide phylogenetic range of teleosts (Breton et al. 1984; Sherwood et al. 1984). Despite differences in their structure, studies on goldfish (*Carassius auratus*) have shown sGnRH and mGnRH to be equipotent in stimulating gonadotropin release in vivo (Peter et al. 1985) and in vitro (MacKenzie et al. 1984). However, the possibility exists that analogues of the native salmon peptide may be more effective in stimulating gonadotropin secretion and inducing ovulation in teleosts than analogues of mGnRH. It has been shown that [D-Arg^6,Pro^9-NEt]-sGnRH was the most potent of several mammalian and salmon GnRH analogues on the stimulation of gonadotropin secretion in goldfish (Peter et al. 1985). Whether a similar tructure–activity relationship for GnRH peptides applies to other teleost species remains to be considered.

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We investigated the effects of sGnRH and mGnRH as well as analogues of each molecule on gonadotropin secretion and the induction of ovulation in coho salmon (*Oncorhynchus kisutch*).

Materials and Methods

Adult female coho salmon were obtained from the Capilano Salmon Hatchery following their return migration. Fish were transferred to the West Vancouver Laboratory, Department of Fisheries and Oceans, and held outdoors in 3-m-diameter fiberglass tanks supplied with freshwater at 10°C. Fish were acclimated to laboratory conditions for at least 5 d prior to hormone treatment.

The peptides used in the experiments and the identification code for each peptide are listed in Table 1. mGnRH was purchased from Peninsula Laboratories Inc., San Carlos, California. [D-Arg^6,Pro^9-NEt]-mGnRH (mGnRH-A) was a gift from Syndel Laboratories, Vancouver, British Columbia. The other peptides listed in Table 1 were provided by J. Rivier and W. Vale. The peptides were dissolved in 0.65% saline. Injection volumes of saline in control fish and of peptide solutions were 0.4 mL/kg body wt.

The procedures used for anesthetization, weighing, and tagging of fish are described in Hunter et al. (1978) and Van Der Kraak et al. (1983). Blood samples (1.0 mL) were obtained by puncturing the caudal vasculature with a 21-guage needle attached to a 2.5-mL syringe. Plasma was obtained following centrifugation and stored at −40°C prior to gonadotropin determination by radioimmunoassay (Van Der Kraak et al. 1983). Ovulation was indicated by the release of a stream of oocytes following the application of gentle pressure to the abdomen.

For multiple comparisons between treatment groups, plasma gonadotropin levels were log-transformed prior to testing with one-way analysis of variance and Duncan's multiple range test. Fisher's exact probability test was used to determine differences between the number of ovulated fish in control and peptide treated groups.

Experiment 1 was started on September 28, 1982, which was approximately 6 wk prior to the expected time of ovulation. Fish received intraperitoneal injections of either mGnRH, mGnRH-A, or sGnRH at dosages of 0.02 or 0.2 mg/kg body wt. Blood samples were obtained from eight fish at the time of injection and serially from all fish at 1.5, 24, and 48 h postinjection. Fish were checked for ovulation 10 d after injection.

Experiment 2 was started on October 14, 1982, which was approximately 4 wk prior to the expected time of ovulation. Groups of 10–13 fish received either mGnRH-A, mGnRH-B, sGnRH, sGnRH-A, or sGnRH-B at a dosage of 0.2 mg/kg body wt. An additional group of eight fish received sGnRH at a dosage of 1.0 mg/kg body wt. Blood samples were taken at 3 h and 1, 2, 4, and 8 d postinjection. Fish were checked for ovulation at 2- to 3-d intervals for a total of 14 d.

Results

Experiment 1

Plasma gonadotropin levels were 6.4 ± 1.9 ng/mL in eight fish sampled at the time of injection. Groups of fish receiving mGnRH, mGnRH-A, and sGnRH at dosages of 0.02 and 0.2 mg/kg body wt had significantly higher plasma gonadotropin levels than saline-injected fish at 1.5 h postinjection (Table 2). The gonadotropin release response to both mGnRH and sGnRH was transitory whereas treatment with mGnRH-A resulted in a more prolonged gonadotropin release response. Plasma gonadotropin levels in fish receiving mGnRH (0.02 and 0.2 mg/kg body wt) and sGnRH (0.02 mg/kg body wt) were similar to saline-injected fish at 24 h postinjection. In contrast, fish receiving a high dosage of sGnRH (0.2 mg/kg body wt) had elevated plasma gonadotropin levels at 24 h, but at 48 h postinjection the levels were similar to those in saline-injected fish. Fish receiving mGnRH-A (0.02 and 0.2 mg/kg body wt) had significantly higher plasma gonadotropin levels at 24 and 48 h postinjection than fish receiving mGnRH or sGnRH. Treatment with mGnRH, mGnRH-A, and sGnRH failed to stimulate ovulation by 10 d postinjection.

Experiment 2

Single injections of mGnRH-A, mGnRH-B, sGnRH-A and sGnRH-B induced a significantly higher rate of ovulation than treatment with saline (Fig. 1). Administration of sGnRH at a dosage of 0.2 mg/kg body wt also induced a higher rate of ovulation than a saline injection, although the response was delayed when compared with mGnRH analogue and sGnRH analogue injections. The number of fish that ovulated in response to mGnRH analogues and sGnRH analogues was significantly higher than the response in saline-injected controls on day 8 whereas sGnRH at a dosage of 0.2 mg/kg body wt did not result in a significant increase in the number of fish having ovulated until day 14. The rate of ovulation in fish receiving sGnRH at a dosage of 1.0 mg/kg body and wt control fish was similar over the 14-d sampling period.

Plasma gonadotropin levels in fish receiving sGnRH and analogues of both mGnRH and sGnRH were significantly higher than those in saline-injected controls at 3 h postinjection and remained significantly higher than controls for at least 96 h (Fig. 2). Fish receiving sGnRH at dosages of 0.2 and 1.0 mg/kg body wt had similar plasma gonadotropin levels at 3 and 24 h postinjection. However, at the 48- and 96-h sampling periods, gonadotropin levels were significantly lower in fish receiving the high dosage of sGnRH. Each of the analogue-treated groups had significantly higher plasma gonadotropin levels than sGnRH-injected fish at 24 and 48 h postinjection. Plasma gonadotropin levels were similar in groups receiving mGnRH-A, mGnRH-B, sGnRH-A, and sGnRH-B at 3, 24,and 48 h postinjection. At 96 h, gonadotropin levels in fish receiving mGnRH-A were significantly lower than the levels in fish

TABLE 1. Primary structure and identification code for GnRH peptides tested in this study.

	1	2	3	4	5	6	7	8	9	10
mGnRH	pGlu	His	Trp	Ser	Tyr	Gly	Leu	Arg	Pro	Gly-NH$_2$
mGnRH-A						D-Ala				NEt
mGnRH-B						(imb$_z$1) D-His				NEt
sGnRH							Trp	Leu		
sGnRH-A						D-Ala	Trp	Leu		NEt
sGnRH-B						D-Arg	Trp	Leu		NEt

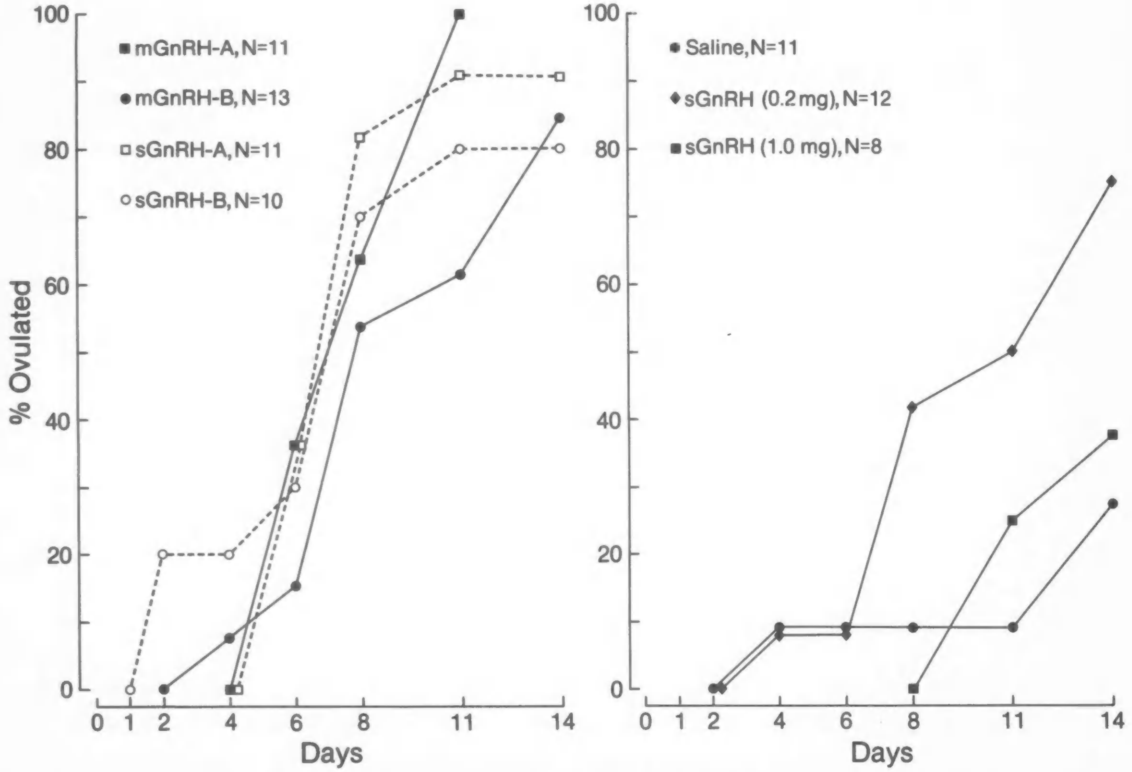

FIG. 1. Cumulative percentage of the number of fish which ovulated in response to sGnRH and analogues of both mGnRH and sGnRH. All peptides were injected intraperitoneally at a dosage of 0.2 mg/kg body wt; an additional group received sGnRH at a dosage of 1.0 mg/kg body wt.

receiving sGnRH-B. At the 8-d sample, gonadotropin levels in each of the peptide-injected groups did not differ significantly from the levels in control fish (data not shown).

Discussion

The present studies demonstrate that sGnRH and mGnRH have similar potencies in stimulating gonadotropin secretion in preovulatory coho salmon (Table 2), which is consistent with the actions of these peptides in goldfish (Peter et al. 1985). The results also confirm an earlier study (Van Der Kraak et al. 1983) by showing that mGnRH-A functions as a superactive agonist in coho salmon by stimulating a more prolonged increase in gonadotropin secretion than either mGnRH or sGnRH. As with mGnRH-A, analogues of sGnRH with D-Arg or D-Ala in position 6 and the Pro^9 -*N*-ethylamide modification of the C-terminus were superactive in stimulating gonadotropin secretion (Fig. 2). Similarly, [(imb$_z$1) D-His^6,Pro^9-NEt]-mGnRH (mGnRH-B) also functions as a superactive GnRH agonist in coho salmon.

The present results differ from those reported by Peter et al. (1985) for goldfish where native peptides and several analogue peptides given as a single intraperitoneal injection were of similar potency in stimulating gonadotropin secretion. Superactivity of GnRH analogues compared with either mGnRH or sGnRH in goldfish could only be demonstrated when these peptides were injected in combination with the dopamine receptor antagonist pimozide. It is known that dopamine

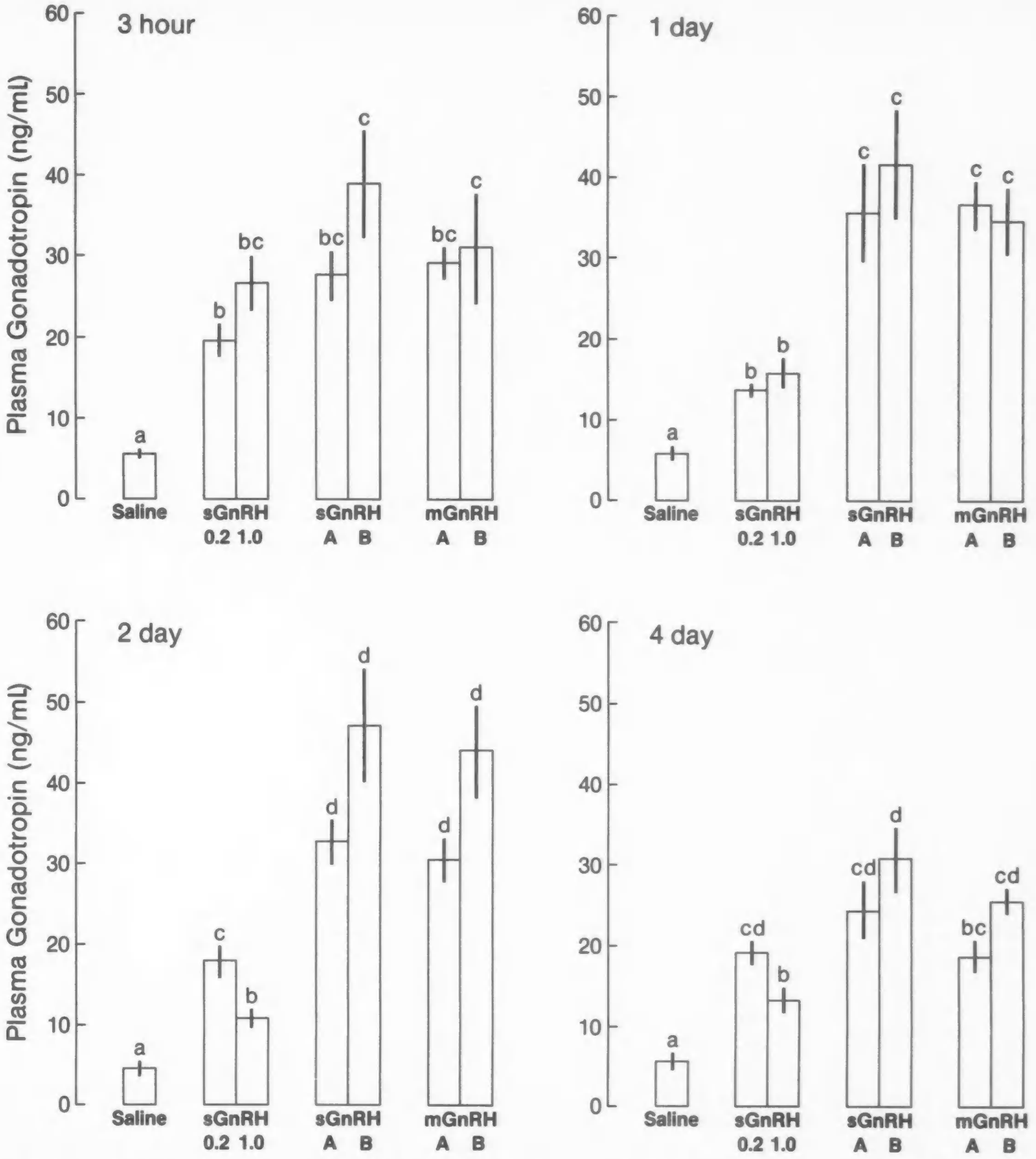

FIG. 2. Effects of sGnRH and analogues of both mGnRH and sGnRH on plasma gonadotropin levels in coho salmon. All peptides were injected intraperitoneally at a dosage of 0.2 mg/kg body wt ($N = 10$); an additional group received sGnRH at a dosage of 1.0 mg/kg body wt ($N = 8$). At each sampling period, treatment groups having similar plasma gonadotropin levels (mean ± SE), as determined by Duncan's multiple range test, are identified by the same letter.

functions as a gonadotropin release-inhibitory factor in several teleost species including coho salmon by modulating spontaneous as well as GnRH-stimulated gonadotropin release (Peter et al. 1986; Van Der Kraak et al. 1986). However, it appears that species differences exist with respect to the relative intensity of the dopamine inhibitory tone (Peter et al. 1986). A reduced intensity of the dopaminergic inhibitory tone on gonadotropin release in coho salmon compared with goldfish may account for differences in the gonadotropin release response to single intraperitoneal injections of GnRH peptides in these species.

In a study that included testing of each of the four GnRH analogues used in the present study, Peter et al. (1985) reported that [D-Arg^6,Pro^9-NEt]-sGnRH (sGnRH-B) was the most potent GnRH analogue in goldfish. In the present study, no

TABLE 2. Effects of mGnRH, mGnRH-A, and sGnRH on plasma gonadotropin levels and ovulation in coho salmon. At each sampling period, plasma gonadotropin levels (mean ± SE) which are similar ($P > 0.05$), as determined by Duncan's multiple range test, are followed by the same letter. The number of fish ovulated was determined 10 d after injection.

Treatment	N	Plasma gonadrotropin (ng/mL)			Number ovulated
		1.5 h	24 h	48 h	
Saline	8	6.9±1.2 a	9.2±0.5 a	8.6±1.1 ab	0
mGnRH					
0.02[a]	8	16.5±1.7 bc	7.0±1.0 a	8.7±1.0 ab	1
0.2	8	19.5±2.4 bcd	7.2±0.5 a	7.8±0.7 ab	0
mGnRH-A					
0.02	8	21.3±1.5 cd	26.7±2.3 c	26.5±3.5 c	2
0.2	7	26.0±1.5 d	35.6±6.4 c	35.5±5.2 c	1
sGnRH					
0.02	5	14.2±0.9 b	8.0±0.9 a	6.4±0.7 a	0
0.2	7	24.8±4.0 cd	14.2±2.4 b	11.3±1.6 b	1

[a] Injected dosage (mg/kg body wt).

major differences were found in the effects of the four GnRH analogue peptides on the stimulation of gonadotropin secretion in coho salmon (Fig. 2). However, the failure to detect differences in the potency of these peptides may reflect testing at a high dosage which elicits a near maximal response; further tests using a graded series of dosages of these analogues are necessary. Whereas sGnRH-A at a dosage of 0.2 mg/kg body wt was superactive in coho salmon, this analogue at a dosage of 0.1 mg/kg body wt tested alone and in combination with pimozide was not superactive in goldfish (Peter et al. 1985). This suggests that the factors which contribute to the superactivity of GnRH peptides in coho salmon differ from those in goldfish.

The present results confirm earlier studies on coho salmon by showing that a single injection of mGnRH-A was effective in inducing ovulation when administered up to 1 mo prior to the expected time of ovulation (Fitzpatrick et al. 1984; Van Der Kraak et al. 1985). Each of the GnRH analogues shown to be superactive in stimulating gonadotropin secretion was also effective in inducing ovulation. Previous studies on coho salmon (Van Der Kraak et al. 1985) and other salmonids (Crim et al. 1983a, 1983b) suggest that a prolonged stimulation of gonadotropin secretion in response to GnRH peptides is necessary to advance ovulation when administered about 1 mo prior to the expected time of ovulation. Treatment of coho salmon with sGnRH at a dosage of 0.2 mg/kg body wt also induced a high rate of ovulation although the response time to ovulation was delayed relative to the response to GnRH analogues (Fig. 1). In part, this may relate to the reduced gonadotropin release response to sGnRH compared with GnRH analogues (Fig. 2). Administration of a higher dosage of sGnRH (1.0 mg/kg body wt) results in reduced gonadotropin secretion at 48 and 96 h postinjection compared with sGnRH at a dosage of 0.2 mg/kg body wt and was an ineffective treatment for the induction of ovulation. It is well known that pituitary responsiveness to GnRH is reduced in mammals following exposure to a high dosage of GnRH owing to a down regulation of pituitary GnRH receptors (see Clayton and Catt 1981). Whether a similar mechanism would account for the reduced effectiveness of sGnRH at high dosages in coho salmon or alternately whether sGnRH has direct inhibitory effects on ovarian function remains to be considered.

Ovulation was induced in coho salmon by the administration of GnRH peptides about 1 mo prior to the expected time of ovulation whereas treatments commencing 2 wk earlier were ineffective. It is likely that changes in the steroidogenic capacity of the ovary contribute to this phenomenon. The ability of the ovary to produce $17\alpha 20\beta$-dihydroxy-4-pregnen-3-one which mediates oocyte maturation develops during the 4- to 6-wk period preceding the normal ovulatory period in salmonids (Young et al. 1983; Van Der Kraak and Donaldson 1986). The present results also suggest that a change in pituitary responsiveness to GnRH contributes to this phenomenon. Whereas the initial magnitude of the gonadotropin response to sGnRH (0.2 mg/kg body wt) was similar in experiments 1 and 2, the response to sGnRH at 24 and 48 h was more prolonged in the second experiment which was conducted closer to the expected time of ovulation (Table 2; Fig. 2). Seasonal differences in pituitary responsiveness to GnRH peptides occur in other salmonids, with responsiveness being greatest during the spawning season (Weil et al. 1978; Crim et al. 1983a; Crim and Glebe 1984). Since the initial response to sGnRH was similar in experiments 1 and 2, it is possible that factors other than the density of GnRH receptors contribute to this phenomenon. These factors could include changes in the intensity of the dopamine inhibitory tone or in steroid hormone feedback effects on the pituitary. Testosterone stimulates accumulation of pituitary gonadotropin and release of gonadotropin to the circulation in juvenile salmonoids (Crim et al. 1981; Crim and Evans 1983). Previous studies have shown a marked elevation of testosterone levels early in the preovulatory period in coho salmon (Van Der Kraak et al. 1984; Fitzpatrick et al. 1986) and a marked increase in the capacity of the ovary to produce testosterone during the 4-to 6-wk period preceding the expected time of ovulation (Van Der Kraak and Donaldson 1986). Further studies have shown that injection of mGnRH-A induces a rapid and prolonged increase in testosterone production in preovulatory coho salmon (Van Der Kraak et al. 1984). On the basis of these results, further studies are warranted to investigate not only the role of testosterone in modulating pituitary responsiveness to GnRH but also the involvement of 17β-estradiol which is decreased during the preovulatory period in coho salmon (Van Der Kraak et al. 1984, 1985; Fitzpatrick et al. 1986).

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Size and Age at Metamorphosis in Marine Fishes: An Analysis of Laboratory-Reared Winter Flounder (*Pseudopleuronectes americanus*) with a Review of Variation in Other Species[1]

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Chambers, R. C., and W. C. Leggett. 1987. Size and age at metamorphosis in marine fishes: an analysis of laboratory-reared winter flounder (*Pseudopleuronectes americanus*) with a review of variation in other species. Can. J. Fish. Aquat. Sci. 44: 1936–1947.

Offspring of adult winter flounder (*Pseudopleuronectes americanus*) collected from Conception Bay, Newfoundland, were reared from fertilization to metamorphosis. We tested two hypotheses: 1) length and age at metamorphosis are equally variable among individuals and (2) length and age at metamorphosis are not correlated. Length at metamorphosis was significantly less variable than age at metamorphosis when data from all laboratory populations were pooled and in 15 of 18 populations. Coefficients of variation for length and age at metamorphosis for the pooled data were 0.051 and 0.123, respectively. Length and age at metamorphosis were positively correlated when the data were pooled ($r = 0.42$, $p < 0.001$) and within 8 of 18 populations. Larvae that metamorphose late do so at larger sizes. When length and age at metamorphosis were converted to growth and developmental rates for the full larval period, significant positive correlations were evident between these rates for the pooled data ($r = 0.68$, $p < 0.001$) and within 16 of 18 populations. Larvae that grow slowly, therefore, remain as larvae longer. An examination of published values on size and age at metamorphosis in marine fishes revealed a pattern consistent with our findings both within and among populations and environments: (1) variation in length was less than variation in age at metamorphosis and (2) positive correlations between growth and developmental rates were evident through the larval period. Increased size at metamorphosis may ameliorate competitive effects and reduce the risk of predation in the period immediately following metamorphosis. Due to the considerable variation in the duration of the larval period (age at metamorphosis) the number of individuals that successfully metamorphose and subsequently enter the mature population could be significantly altered under differing environmental conditions.

La progéniture de plies rouges adultes (*Pseudopleuronectes americanus*) capturées dans la baie Conception (Terre-Neuve) a été élevée du stade de la fécondation à celui de la métamorphose. Les auteurs ont testé deux hypothèses : 1) la longueur et l'âge à la métamorphose sont également variables entre les individus et 2) la longueur et l'âge à la métamorphose ne sont pas corrélés. La longueur à la métamorphose était significativement moins variable que l'âge lorsque les données de toutes les populations de laboratoire étaient regroupées, de même que chez 15 des 18 populations. Les coefficients de variation de la longueur et de l'âge à la métamorphose des données regroupées étaient respectivement de 0,051 et de 0,123. La longueur et l'âge à la métamorphose étaient positivement corrélés lorsque toutes les données étaient regroupées ($r = 0{,}42$, $p < 0{,}001$) de méme que chez 8 des 18 populations. Les larves qui se métamorphosaient plus tard le faisaient aussi à des tailles plus grandes. Lorsque les valeurs de longueur et d'âge à la métamorphose étaient converties en taux de croissance et de développement portant sur toute la période larvaire, il y avait des corrélations positives significatives entre ces taux, ceci pour les données regroupées ($r = 0{,}68$, $p < 0{,}001$) de même que chez 16 des 18 populations. Les larves à croissance lente demeuraient donc plus longtemps au stade larvaire. Un examen des valeurs publiées relatives à la taille et à l'âge à la métamorphose chez les poissons marins a montré l'existence d'une tendance cohérente avec les résultats obtenus, tant au sein des populations et des environnements qu'entre eux à savoir : 1) la variation de la longueur était inférieure à la variation de l'âge à la métamorphose et 2) il y avait, tout au long de la période larvaire, des corrélations positives entre les taux de croissance et de développement. Une taille accrue au moment de la métamorphose peut avoir pour effet d'accroître la compétitivité et de réduire les risques de prédation pendant la période suivant immédiatement la métamorphose. A cause de la variation de la durée de la période larvaire (âge à la métamorphose), le nombre d'individus qui se métamorphosent et se joignent ensuite à la population mature pourrait être influencer de façon appréciable par differentes conditions environnementales.

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In the typical marine fish life cycle an individual not only passes through a wide range of body sizes but also through several discrete life periods. Both pelagic and demersal embryos normally hatch into active pelagic larvae. The larvae subsequently metamorphose into juveniles that are of the adult form. Associated with these morphological transitions are important ecological and behavioral changes.

Estimates of mortality rates can be conveniently and justifiably related to these discrete periods of the life cycle. These rates are extremely high in the egg and larval periods (McGurk 1986). Both the passive embryos and active larvae are vulnerable to predation, and the risk of starvation is maximal early in the larval period (Hewitt et al. 1985). Mortality rates as high as $0.4\text{–}0.8 \cdot d^{-1}$ are common in early larval life (Fortier and Leggett 1985; Hewitt et al. 1985; Ware and Lambert 1985; Taggart 1986). The view that interannual variation in mortality rates in early life is the principal regulator of recruitment variability in these species is a cornerstone of the current view of fish population dynamics (Gulland 1965; Shepherd et al. 1984; van der Veer 1986). This view has motivated extensive field and laboratory work on causes, magnitude, variability, and correlates of mortality during the embryonic and early larval periods. Laboratory studies have typically focused on the embryonic through the yolk-sac period. The responses measured in both field and laboratory studies are, with few exceptions, survival, size at age, and size and age at hatching. Only infrequently the observed extreme, modal, or average size and age at metamorphosis are reported for field (e.g. Minami 1984) or laboratory (Shelbourne 1964; Blaxter 1968; Laurence 1975; Kuronuma and Fukusho 1984; Seikai et al. 1986) populations. In species where metamorphosis is relatively gradual and difficult to score, such as in the clupeoids or gadoids, metamorphosis has been operationally defined as the attainment of a set size (e.g. Hunter and Kimbrell 1980; Sinclair and Tremblay 1984; Bailey and Stehr 1986). This convention precludes analysis of the variance in and correlation between size and age at metamorphosis and may account for the lack of attention on the terminus of the larval period that is signalled by metamorphosis.

In this paper we report the results of studies of phenotypic variation and correlation in sizes and ages at metamorphosis in laboratory populations of winter flounder (*Pseudopleuronectes americanus*). We view winter flounder as a model for marine fish that have a pelagic to demersal habitat transition associated with acquisition of juvenile characters (metamorphosis). Because staging of flatfish larvae is easily and commonly done (Shelbourne 1957; Ryland 1966; Seikai et al. 1986), the flatfish as a group are well-studied among marine fish species (Ahlstrom et al. 1984), thereby providing a data base with which we compare our results.

We pose two questions. First, what is the relative magnitude of the variation in size and age at metamorphosis and is the pattern consistent among populations, environments, and species? This issue has been cast in a more causal form by Policansky (1982): is metamorphosis "influenced more by size or age?" Although there must be specific size and age boundaries on the metamorphic process set proximally by a physiological response to and production of inductive hormones (e.g. Inui and Miwa 1985), we view size and age as characters that describe an individual at metamorphosis rather than invoking a dependency of metamorphosis on these characters. Implicit in this perspective and indeed central to our work is the expectation of variation among individuals for these characters.

Second, are size and age at metamorphosis correlated? If size and age at metamorphosis are correlated, then prediction of one is possible from estimates of the other. The ability to predict age from size is important because age data for larval fish, while obtainable, are technically demanding and costly. Conversely, if estimates of age are available, then size may be predicted. For example, the duration of a life period is known to vary inversely with temperature in both the embryonic (Rogers 1976; Harding et al. 1978) and larval periods (Laurence 1975; Seikai et al. 1986). Estimates of temperature-dependent larval period duration and of the correlation between age and size at metamorphosis make size at metamorphosis in natural populations predictable under given temperature regimes.

Size and age at a life period transition, such as metamorphosis or maturation, are reflections of growth and developmental rates up to that point. The distinctions between growth, development, size, and age have not consistently been made in the marine fish literature. Some authors, as mentioned above, have pragmatically defined metamorphosis by a size criterion, and others (e.g. Smith 1985) equate life period duration with the reciprocal of growth rate. Growth is the change in size with age; development is differentiation and maturation of cells, tissues, and organ systems. Developmental rate may be operationally defined as the inverse of age at transition. It seems intuitive that growth and developmental rates should be positively correlated but there are exceptions. In organisms where transitions are associated with age, developmental rate is effectively independent of growth rate. Examples include maturation in pink salmon (*Oncorhynchus gorbuscha*), where nearly all fish mature at 2 yr (Hart 1973), and pupation in blowflies (*Chrysomyia albiceps*), where Ullyett (1950) found overcrowding to decrease pupal mass by 57% without altering the duration of the larval period.

Methods

Species Studied

Winter flounder is a right-eyed flounder (family Pleuronectidae) found in shallow coastal waters from Georgia, USA, to Newfoundland and Laborador, Canada. Adults migrate inshore to spawn in shallow bays and estuaries. It is exceptional among Atlantic flatfish in that it spawns in late winter or early spring (January through June, depending on latitude) and its eggs are demersal and adhesive. These reproductive characters, shared with four other western Pacific pleuronectines, are viewed by Pearcy (1962d) as an adaptation for exploiting marine shallows early in the season when ice may still be covering the estuaries. Rogers (1976) reported that the embryonic period lasts 5–31 d depending on water temperature. Hatchlings spend the first 4-14 d of life without exogenous feeding, absorbing nutrients from their yolk sac. Larvae begin to feed at or shortly after absorption of their yolk (Buckley 1982). Nauplii, copepods, polychaetes, and invertebrates eggs were dominant components of the diet, and prey size increased with the size of larvae in an estuarine population of winter flounder larvae in Connecticut, USA (Pearcy 1962c). The rate of growth of larvae is exponential in mass (Laurence 1977). Metamorphosis, which includes migration of the left eye to the right side of the head and loss of pigmentation on the blind left side, occurs from 7 to 13 mm standard length in natural and laboratory populations and from 6 to 14 wk after first feeding in laboratory, field enclosure, and natural populations (Bigelow and Schroeder 1953; Laurence 1975; Laurence et al. 1979; Fahay 1983). The morphological metamorphosis is coupled with a major ecological shift. During the approximate 1-wk period of rapid migration of the left eye the fish progressively switch from pelagic to demersal habitat and feeding behavior. Juveniles commonly spend their first 2–3 yr in estuarine shallows, with seasonal departure to deeper waters to avoid cold temperatures nearshore. Adults have a narrower range of temperature tolerance than juveniles and occupy deeper waters of estuaries and bays. Age at maturation varies directly with latitude from 2 to 9 yr and length at maturation varies from 17 to 30 cm total length. The life cycle, ecology, and bionomics of winter flounder are thoroughly reviewed in Klein-McPhee (1978).

Experimental Rationale

We approached our questions from a laboratory setting because we wanted measures of variances in sizes and ages of fish at metamorphosis with minimal environmental effects and measurement error. We have not applied any experimental treatment to the reared larvae except that populations of larvae differed in parentage and were reared in full-sib groups. Temperature (Laurence 1975; Policansky 1982; Crawford 1984; Seikai et al. 1986), food level (Laurence 1975; Houde 1977; Houde and Schekter 1981), food type (Howell 1973), and intitial density of larvae (Houde 1977) are known to alter growth and/or developmental rates in experimental populations of flatfish larvae. These and other environmental factors could have profound effects on the distributions of characters we assessed.

The potential disadvantage of the laboratory approach is that growth and developmental rates may be abnormal. Conclusions from comparisons of laboratory and field larvae are equivocal, varying among species and investigators. Houde (1971) noted a number of developmental abnormalities in laboratory-reared lined soles (*Achirus lineatus*) although these had little affect on growth rate. Ehrlich (1975) and Buckley (1981) found field-grown larvae of European plaice (*Pleuronectes platessa*) and winter flounder, respectively, to be 1.5–2.0 times heavier (dry weight) for a given length than laboratory-reared larvae. Riley (1966) showed, however, that laboratory-reared larvae of European plaice had a higher mass both for age and for larval stage than field specimens. Fukuhara (1983) reported that laboratory-reared larvae of Japanese anchovy (*Engraulis japonica*) were longer at age and longer at metamorphosis than larvae from field populations. Houde et al. (1970) found comparable or greater differences between juveniles from two disjunct field populations than between either of the two and laboratory-reared animals in morphometric characters corrected for differences in body length. The value of comparisons of larvae from laboratory and natural populations would be increased if potentially confounding factors, such as differences in type and duration of preservation, source populations, and dominant environmental factors such as temperature, were accounted for or controlled. Our data on growth rates and lengths and ages at metamorphosis are in agreement with other laboratory (Laurence 1975; Radtke and Scherer 1982), enclosure (Laurence et al. 1979), and field values (Pearcy 1962b; Crawford and Carey 1985) for winter flounder. We feel justified in using data from laboratory-reared larvae as a first approximation to growth and development expressed under natural conditions, particularly given that our interests are in patterns of variance and correlation rather than a strict reliance on the actual values of lengths and ages observed. Nonetheless, we encourage studies that are conducted so that laboratory and natural populations can be fairly compared.

Laboratory Methods and Analysis

Adult winter flounder were collected by gill netting and SCUBA diving in late May 1986 at Bryant's Cove, Newfoundland (47°40.6′N, 53°11.4′W). Laboratory facilities were at the same location. Males and females were kept separated in a flow-through tank at approximately 6°C where they ripened naturally. Sufficient adults were collected to ensure that the required number of females could be mated within a narrow time period (10–14 June). This minimized any uncontrolled temporal variation in the rearing environment.

Each of four males was crossed with two unique females. Sperm was extruded into a 20-mL vial for each male and kept in an ice bath until eggs were prepared. Eggs from ripe females were extruded onto a chilled plastic pan (40 × 25 × 8 cm). Sperm was added by disposable pipette to eggs and the pan swirled to distribute eggs over the pan bottom in a monolayer. This ensured a high fertilization rate. After 2 min, a well-mixed suspension of diatomaceous earth (50 g . L) was poured over the fertilized eggs and the pan was rocked vigorously for 5 min. This solution prevents most eggs from clumping to one another (Smigielski and Arnold 1972).

Fertilized eggs were kept at 5°C throughout the incubation period. At 5 h after fertilization the eggs were separated from the diatomaceous earth by pouring the contents of the spawning pan through 560-μm netting. The netted eggs were rinsed with filtered, UV-sterilized seawater and temporarily divided into two incubation pans per family. At 2 d after fertilization, three groups of 500 eggs per female were placed into 400-mL polyethylene dishes for the remainder of the embryonic period. The water in the egg dishes was changed every second day. At the second water change the eggs received a treatment of streptomycin ($0.02\ \mathrm{mg \cdot mL^{-1}}$) and penicillin G ($25\ \mathrm{IU \cdot mL^{-1}}$). Twice during the embryonic period the egg dishes were inspected and dead embryos were removed. At 1–3 d prior to hatching, 400 late-eyed embryos from each incubation dish were placed into hatching dishes that were floated in each of the 24 rearing aquaria. The hatching dishes had openings which allowed the hatchlings to swim directly out into their aquarium, thus avoiding handling.

Larvae were reared in 40-L glass aquaria. The walls of each aquarium were covered externally with black plastic to enhance the constrast between prey items and the tank background. The rearing water was filtered to 0.45-μm and sterilized with UV light. Rearing conditions were static except for partial water replacement of approximately 250 mL at daily food additions. An antibiotic treatment (as detailed above) was applied to each aquarium at 2 wk after hatching. Light aeration was applied to each aquarium at 4 wk after hatching. The bottom of each aquarium was swept with gravity suction every 4–5 d. Chilled water baths maintained all aquaria at 6.9 ± 0.83°C (mean ± SD). Light was provided at a distance of 0.75–1.5 m above each aquarium with two 40-W fluorescent bulbs. A 14 h : 8 h light–dark photoperiod was maintained throughout the study.

Larvae were fed in excess through the larval period. Laboratory-cultured rotifers (*Brachionus* sp.) were added bidaily through week 3 and daily during weeks 4 and 5 to give a prey density of $4.2 \pm 1.37 \cdot \mathrm{mL^{-1}}$. The rotifers were cultured on either green (*Dunaliella* sp. or *Platymonas* sp.) or golden

(*Isochrysis* sp.) algae and concentrated by siphoning the contents of culture aquaria through 20-μm netting. The netted rotifers were placed in dense algal cultures (10^5–10^6 cells·mL^{-1}) and chilled for 6–12 h prior to inoculation into the larval aqauria. Starting at 5 wk after first feeding the larvae were fed daily with field collected zooplankton at 2.0 ± 0.83·mL^{-1}. Zooplankton were collected by towing a 1-m-diameter, 80-μm-mesh plankton net at <1 m·s^{-1} for 5 min. Only zooplankton that passed through a 183-μm mesh and collected on 41-μm-mesh netting were used as a source of food. At 6 wk after first feeding the size fraction of zooplankton used was elevated to a range of 41–335 μm. The primary constituents of both size fractions were larval copepods but trochophores were also common. Brine shrimp (*Artemia* sp., Chinese var.) and rotifers were used as an auxillary food when the weather prevented plankton tows or natural zooplankton were sparse (10 of 30 d).

Aquaria were checked daily as the larvae approached metamorphosis. Metamorphosis was operationally defined as the point at which the iris of the migrating eye was visible from the right side of the body. This is equivalent to stage 5 of Shelbourne (1957) and Ryland (1966) and stage H of Seikai et al. (1986). These animals, referred to hereafter as metamorphs, were anaesthetized in chilled MS-222 (25 mg·mL^{-1}) and total lengths (TL) measured with an ocular micrometer at magnification of 10 ×. After reviving, metamorphs were returned to new aquaria holding only juveniles that had shared an aquarium as larvae. Age at metamorphosis was defined as the number of days between scoring a fish as a metamorph and the modal hatching day for larvae from its aquarium. Growth and developmental rates were derived from length and age at metamorphosis by

$$\text{Growth rate} = \frac{(\text{TL at metamorphosis} - \text{average TL of hatchlings})}{\text{age at metamorphosis}}$$

and

$$\text{Developmental rate} = (\text{age at metamorphosis})^{-1}.$$

Metamorphs with abnormal eye migration, although infrequent ($<2\%$), were excluded from the analysis. Rearing was terminated and all fish counted and measured during 3–5 September at an age of 10 wk after hatching.

Only aquaria that had a minimum of 10 individuals at the termination of the rearing were included in the analysis. This restriction yielded populations from 18 aquaria. The means and variances for sizes and ages at metamorphosis that we report are slight underestimates because 18 % of the flatfish that survived to the termination date had not yet metamorphosed. These larvae were not distributed uniformly among aquaria.

The relative variation for lengths and ages at metamorphosis was assessed by comparing their respective coefficients of variation (CV). This is a measure of variation standardized for differences in averages and, in our case, units of measure. Although CVs are dimensionless, their values depend on the measurement used. If, for example, size is quantified by mass rather than length, then the CV of mass would be proportional to the CV of length with the proportionality constant equaling the exponent that is used to convert length to mass. We used the bias-correcting formula proposed by Haldane (1955) for calculating the CVs:

$$\text{CV}' = (1 + 1/(4n))\cdot\text{CV}$$

where n is the sample size and CV is computed as the standard deviation divided by the mean. The test statistic for significant differences between the CVs for length and age at metamorphosis was calculated as

$$\frac{\text{CV}'_1 - \text{CV}'_2}{\sigma_{\text{CV}'_1 - \text{CV}'_2}}$$

where

$$\begin{aligned}\sigma_{\text{CV}'_1 - \text{CV}'_2} = \{&(1 + 1/4n)^2(1/4n)\\ &\times [\text{CV}'^2_1(n/(n-1) + 2\text{CV}'^2_1)\\ &+ \text{CV}'^2_2(n/(n-1) + \text{CV}'^2_2)\\ &- 2\text{CV}'_1\text{CV}'_2 r(n/(n-1)r + 2\text{CV}'_1\text{CV}'_2)]\}^{1/2}.\end{aligned}$$

The subscripts of CV′ reference the two variables being compared (length and age); r is the Pearson product-moment correlation between the two variables. This formula is appropriate when variables being compared are interdependent, as is expected in the bivariate measure of length and age at metamorphosis of an individual. This statistic is tested against a t-distribution with $2(n - 1)$ degrees of freedom (Sokal and Braumann 1980).

Results

A total of 662 flatfish from 18 aquaria (hereafter referred to as populations) were reared through metamorphosis. Summary statistics, correlations, and variance tests by population are given in Table 1. Length and age at metamorphosis were distributed in a conspicuously different manner (Fig. 1). Length at metamorphosis, ranging from 6.6 to 10.1 mm TL, was normally distributed around an average value of 7.8 mm. The symmetry of this distribution suggests that bias due to early termination of the rearing was minimal. The distribution of age at metamorphosis was platykurtic relative to the distribution of length at metamorphosis (Fig. 1). The average age at metamorphosis was 59.5 d from hatching (Table 1). The within-population range of ages at metamorphosis averaged 20.1 d and was as great as 25 d. The proportion of larvae remaining at the termination of the experiment, ranging from 1.6 to 57.6%, was uneven across populations (Table 1). Terminating the rearing after all fish had metamorphosed would have resulted in an increase in the overall average and variance in ages at metamorphosis and an increase in the among-aquaria variance in ages at metamorphosis. Even though the variance in age at metamorphosis was underestimated, length at metamorphosis was significantly less variable than age at metamorphosis when all observations were included ($\text{CV}' = 0.051$ and 0.123, respectively; Table 1; Fig. 1). Length was significantly less variable than age at metamorphosis in 15 of 18 populations. The relative magnitude of the CVs in the remaining three populations also reflected this pattern, but statistical significance was limited by the small sample size (Table 1).

The average length at hatching, used to calculate growth rate, was 3.83 mm TL. The average growth rate from hatching to metamorphosis for all observations was 0.068 (SD = 0.008) mm·d^{-1} (Table 1). Growth rates, calculated for each population at biweekly intervals, were as high as 0.12 mm·d^{-1}. Overall, growth rates were highest in the fourth through eighth weeks of rearing.

The correlation between length and age at metamorphosis was significantly positive when all observations were pooled

TABLE 1. Summary statistics for length and age at metamorphosis and growth and developmental rates of laboratory-reared winter flounder (*Pseudopleuronectes americanus*). Average values (SD) are presented by population. Pop. = population, m = number of metamorphs, l = number of larvae at termination of rearing, r_{la} = correlation coefficient between length and age, r_{gd} = correlation coefficient between growth and developmental rates, CV′ = coefficient of variation, t = t-statistic. The test for differences between the CVs is presented in the text. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Pop.	m	l	Length at metamorphosis[a]	Age at metamorphosis[b]	r_{la}	Growth rate[c]	Developmental rate[d]	r_{gd}	CV′ length	CV′ age	t
1	61	1	7.57 (0.35)	57.4 (6.05)	0.21	6.58 (0.87)	1.75 (0.17)	0.72***	0.046	0.105	5.65***
2	90	6	7.55 (0.37)	54.7 (6.70)	0.24*	6.88 (0.98)	1.85 (0.21)	0.71***	0.050	0.123	7.38***
3	32	5	7.72 (0.32)	57.0 (8.67)	0.48**	6.94 (0.95)	1.79 (0.27)	0.85***	0.043	0.153	5.66***
4	119	15	7.80 (0.34)	61.0 (7.65)	0.38***	6.59 (0.80)	1.66 (0.21)	0.76***	0.044	0.126	9.65***
5	47	31	8.01 (0.26)	66.2 (4.19)	0.02	6.34 (0.56)	1.51 (0.26)	0.71***	0.033	0.064	3.99***
6	34	4	7.79 (0.39)	57.8 (7.94)	0.58***	6.92 (0.77)	1.76 (0.23)	0.73***	0.050	0.138	5.34***
7	63	25	8.00 (0.29)	60.3 (6.85)	0.11	6.97 (0.87)	1.67 (0.19)	0.84***	0.036	0.114	7.13***
8	12	2	7.83 (0.28)	56.7 (7.79)	0.79***	7.13 (0.62)	1.79 (0.24)	0.86***	0.037	0.140	3.83***
9	9	2	7.96 (0.20)	60.7 (6.11)	0.37	6.85 (0.68)	1.66 (0.17)	0.88**	0.026	0.103	2.88*
10	15	2	8.03 (0.34)	64.0 (2.91)	0.27	6.58 (0.67)	1.56 (0.07)	0.64**	0.043	0.046	0.24
11	13	1	7.94 (0.33)	56.6 (5.67)	0.61*	7.29 (0.58)	1.78 (0.17)	0.62*	0.043	0.102	2.98**
12	10	2	7.95 (0.32)	60.9 (5.10)	0.57	6.79 (0.50)	1.65 (0.14)	0.52	0.042	0.086	2.18*
13	49	10	7.99 (0.44)	61.6 (5.86)	0.45***	6.78 (0.70)	1.63 (0.17)	0.49***	0.056	0.096	3.86***
14	53	7	7.98 (0.38)	59.5 (7.35)	0.18	7.08 (1.00)	1.70 (0.22)	0.78***	0.048	0.124	5.76***
15	19	1	7.70 (0.34)	56.3 (5.78)	0.42	6.92 (0.72)	1.79 (0.18)	0.60**	0.045	0.104	3.29***
16	14	19	8.35 (0.34)	63.2 (4.17)	0.07	7.18 (0.74)	1.58 (0.11)	0.67**	0.042	0.067	1.62
17	8	4	8.23 (0.32)	65.5 (4.44)	0.28	6.75 (0.76)	1.53 (0.11)	0.76*	0.041	0.070	1.32
18	14	1	7.65 (0.54)	57.7 (7.31)	0.85***	6.62 (0.49)	1.75 (0.22)	0.08	0.073	0.129	3.01**
Total	662	138	7.83 (0.40)	59.5 (7.34)	0.421***	6.79 (0.84)	1.71 (0.21)	0.68***	0.051	0.123	20.70***

[a] Total length (mm). [b] Age (d) since hatching. [c] Growth rate × 100.
[d] Developmental rate × 100.

(Table 1; Fig. 1). This means that fish that metamorphose late do so at significantly larger sizes. The positive correlations between length and age at metamorphosis had significant within and among-population components. Eight of 18 correlations within populations were significantly greater than zero. The correlations from the remaining 10 populations did not significantly depart from zero but they were all in the positive direction. The correlation among population means was significant ($r = 0.79$, df = 16, $p < 0.001$).

The overall correlation between growth and developmental rates to metamorphosis was larger than that between length and age at metamorphosis (Table 1; Fig. 2). The correlations within populations were significantly greater than zero in 16 of 18 cases. The remaining two correlations were not significantly different from zero but were also positive. The among-population correlation between average growth and developmental rates was positive although not significant ($r = 0.43$, df = 16, $p < 0.07$).

The positive correlations between growth and developmental rates and between length and age at metamorphosis are not redundant. They indicate that fish with lower daily growth rates during the larval period metamorphosed later than fish with higher growth rates, even though these later fish were larger at metamorphosis (as reflected in the positive correlation between length and age at metamorphosis). Conversely, fish with rapid growth rates metamorphosed early (i.e. had a shorter larval period) but at a small size.

No density effect on the metamorphic characters was detected. Although all populations began with the same initial number of embryos, survival to the end of the experiment was variable among populations (Table 1). The correlations among populations between the final number (metamorphs plus larvae) and the average size ($r = -0.26$) or age ($r = 0.10$) at metamorphosis were both nonsignificant.

Discussion

Given the consensus on the importance of the embryonic and larval periods to population dynamics of marine fishes, it is surprising that so few studies have reported patterns of sizes and ages of fish as they exit the larval period. Length at metamorphosis is reported for most well-known species in ichthyofaunal surveys (e.g. Fahay 1983), but the intraspecific variance in this character is largely ignored. Age at metamorphosis, rarely reported in species accounts, is more problematic. Ages at metamorphosis are estimated in most autecological studies by subtraction of the estimated date of spawning from the date at collection of metamorphosing larvae. These estimates from field collections should be considered as rough approximations for most species, since the actual date of spawning is unknown, potentially falling within a several-month interval (Pearcy 1962a; Crawford and Carey 1985). Furthermore, the timing of metamorphosis is obscured by commonly used staging schemes (Shelbourne 1957; Ryland 1966; Doyle 1977; Seikai et al. 1986). Laboratory rearing studies of marine fish larvae compromise realism for accuracy in monitoring growth and developmental rates. Ideally, continuity between laboratory and field populations can be established through intermediate levels of intervention such as in situ enclosures (e.g. Laurence et al. 1979; Frank and Leggett 1986; de Lafontaine and Leggett 1987a, 1987b). Greatly improved estimates of larval period duration in natural populations are being provided by daily rings on otoliths in species that have a change in otolith structure

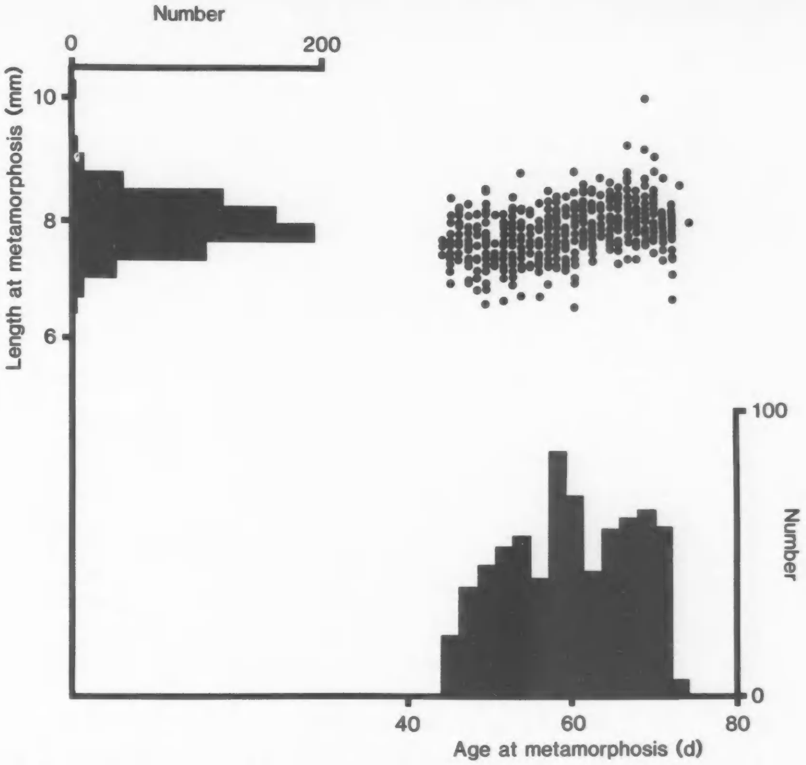

FIG. 1. Lengths and ages at metamorphosis in laboratory-reared winter flounder (*Pseudopleuronectes americanus*). All observations are included in the histograms and the bivariate plot ($N = 661$). Each point in the bivariate plot represents the length and age of an individual at metamorphosis. Some plotted points represent more than one individual.

corresponding to the timing of metamorphosis (Rosenberg and LaRoche 1982; Brothers et al. 1983; Victor 1986).

Within-Environment Patterns in Size and Age at Metamorphosis

Our results on fish reared in unmanipulated environments show variation in length at metamorphosis to be significantly less than the variation in age at metamorphosis both within and among populations. Furthermore, these two characters are positively correlated and, when converted to growth and developmental rates of individuals, are more tightly coupled. Previous references to the variance in and correlation between size and age at metamorphosis within populations of marine fishes range from anecdotal to experimental observations. Ford (1930) observed that the length at metamorphosis in herring (*Clupea harengus*) is variable within stocks and tends to decrease as the spring progresses. Shelbourne et al. (1963) noted that in laboratory-reared European plaice "some pelagic forms grow faster and metamorphose sooner than other." Ryland (1966), also working with laboratory populations of European plaice, stated that "growth and development proceed together in a regular manner" and "larvae late to reach a given stage have nevertheless attained the appropriate weight by that time."

The only other published data on sizes and ages at metamorphosis of *individual* fish are Policansky's (1982, 1983) laboratory studies of the starry flounder (*Platichthys stellatus*) and Victor's (1986) work on settlement characteristics in natural populations of the bluehead wrasse (*Thalassoma bifasciatum*). Policansky reared laboratory populations of starry flounder from several crosses in nine different aquaria. He monitored the rearing temperature and intentionally varied it in one tank. Otherwise, his rearing protocol was similar to ours including daily inspection for metamorphs and recording of their lengths and ages. Victor collected and measured wrasses that were newly settled (<24 h) to the coral reef habitat of the juveniles and adults. He measured lengths of fish at capture and estimated their ages at settlement from the number of rings on the sagittal otoliths. Evidence is provided in Victor (1982) that these rings are indeed daily.

Policansky's (1982) data show length at metamorphosis to be less variable than age at metamorphosis within populations. This agrees with our results on winter flounder. The average CVs for length and age at metamorphosis, calculated from table 1 of Policansky (1982), were 0.056 and 0.132, respectively. These values are close to our values of 0.051 and 0.123 for winter flounder, despite a 2–3°C temperature range in Policansky's rearings. The CVs by population consistently reflect this pattern of less variation in length than age at metamorphosis (Table 2).

Policansky's results on the correlation between length and

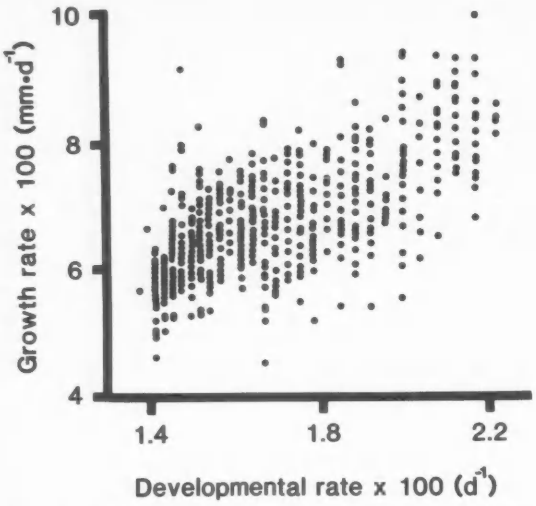

FIG. 2. Bivariate plot of the growth and developmental rates in laboratory-reared winter flounder (*Pseudopleuronectes americanus*) during the larval period. Growth rate is the average daily increment in total length over the larval period. Developmental rate is the reciprocal of age at metamorphosis. All observations are included in the plot (N = 661). Some plotted points represent more than one individual.

TABLE 2. Relative variation in length and age at metamorphosis in starry flounder (*Platichthys stellatus*) and bluehead wrasses (*Thalassoma bifasciatum*). m = number of metamorphs, CV′ = coefficient of variation, t = t-statistic. The test for differences between the CVs is presented in the text. *$p < 0.01$; **$p < 0.001$.

Population		m	CV′ length	CV′ age	t
Starry flounder[a]	1	487	0.059	0.134	15.688
	2	127	0.052	0.148	9.636
	3	364	0.052	0.141	15.908
	4	186	0.071	0.136	8.426
	5	165	0.051	0.128	9.882
	6	28	0.054	0.108	3.254
	7	173	0.047	0.096	8.499
	8	90	0.051	0.132	7.521
	9	56	0.065	0.166	5.781
Bluehead wrasse[b]	1	45	0.063	0.120	4.889

[a] Calculated from Policansky (1982, table 1).

[b] Calculated from Victor (1986, fig. 4B).

age at metamorphosis of starry flounder show a pattern different from ours. Policansky found the within-aquarium correlation to be significantly less than zero in four of nine cases and the correlation was not significant in the remaining five. The correlation between average lengths and ages at metamorphosis among aquaria, calculated from table 1 of Policansky (1982), is significantly less than zero ($r = -0.74$, df = 7, $p < 0.05$), but this is due to inclusion of the one significantly warmer aquarium. Omitting the warm aquarium nullifies the significant correlation. The correlation between growth and developmental rates among populations, calculated from average values given in table 1 of Policansky (1982), was significantly positive with ($r = 0.989$, df = 7, $p < 0.001$) or without ($r = 0.943$, df = 6, $p < 0.001$) the warm aquarium. The within-population correlation between growth and developmental rates was positive for the data displayed in fig. 1–3 of Policansky (1982). These starry flounder have a stronger positive coupling between growth and developmental rates than we found in winter flounder.

Victor's (1986) data on wrasses are also in agreement with our data on winter flounder. They too show less variation in length than in age at metamorphosis. The CVs for length and age at settlement are 0.063 and 0.120, respectively (Table 2). These values are surprisingly similar to those for winter and starry flounder, given that Victor's data are from natural populations, ages are based on otolith increments, and wrasses are distantly related to the flatfish studied by us and Policansky (Tables 1, 2).

Length and age at metamorphosis were positively correlated in wrasses ($r = 0.67$, df = 43, $p < 0.001$). There was also a significant positive correlation between growth and developmental rate when our method of calculation is used ($r = 0.81$, df = 43, $p < 0.001$). Victor (1986) observed, however, that growth rates during the larval or precompetency period showed no significant differences between wrasses from a group with "average" larval duration and a group with "extended" larval duration. Victor defined the precompetency period as the period recorded on the otoliths prior to a transition zone where distances between daily ototith increments are reduced. He concluded that protraction of the larval period occurred in some larvae after they achieved the minimum size for metamorphosis. This was associated with a subsequent reduction in growth rate. Delayed metamorphosis has not been documented for any other species of marine fish, and we found no evidence of a delay switch in our reared winter flounder. Pearcy et al. (1977) noted, however, unusually large larvae of Dover sole (*Microstomus pacificus*) from natural populations. These were believed to be part of a residual population of larvae that had not yet been induced to metamorphose. Facultative delay of metamorphosis is known for a variety of marine invertebrate taxa (Pechenik 1986).

Other Laboratory Studies: Environmental Effects on Size and Age at Metamorphosis

A small number of laboratory studies have reported the effect of different rearing environments on size and/or age at metamorphosis and growth through the larval period. The data provided are not sizes and ages of individuals but rather population or environmental averages, usually with an associated dispersion statistic. By giving a measure of the plasticity of metamorphic characters across rather than within environments, these data may more accurately reflect the potential plasticity of growth and development of larvae expressed in natural populations. Most commonly, temperature is varied across populations (Laurence 1975; Policansky 1982; Crawford 1984; Seikai et al. 1986) although food level (Riley 1966; Laurence 1977; Houde 1977; Houde and Schekter 1981), food type (Howell 1973), crowding of larvae (Houde 1977), and various rearing conditions (Shelbourne 1964) have also been systematically manipulated.

Reduced temperature consistently results in reduced developmental rate (increased age at metamorphosis) and reduced daily growth rate, but the effect on length at metamorphosis is not consistent among studies (Table 3). Laurence (1975) reared winter flounder larvae at 2, 5, and 8°C to determine the effects of temperature on their growth and metabolic rates. He did not

TABLE 3. Changes in lengths and ages at metamorphosis in flatfish due to rearing under different temperatures. Length and age changes are proportional differences in the metric with decreasing temperatures using the warmest temperature as the standard.

Species (source)	Temperature (°C)	Length at metamorphosis (mm)	Length change	Age at metamorphosis (d)	Age change
Pseudopleuronectes	2	—	—	180[b]	
americanus					3.67
	5	8.21 SL		80	
(Laurence 1975)[a]			0.93		1.63
	8	8.82 SL		49	
Platichthys	10	7.09 SL[c]		65[c]	
stellatus			0.92		1.67
(Policansky 1982)	12	7.72 SL		39	
Palalichthys	13	13.5 TL[d]		70[e]	
olivaceus			1.23		2.59
(Seikai et al. 1986)	16	12.4 TL[d]		38[e]	
			1.13		1.41
	19	11.0 TL[d]		27[e]	
Ammotretis	12.7–16.5	11.2 TL		69	
rostratus			1.05		1.92
(Crawford 1984)	16.0–17.4	10.7 TL		36	
Rhombosolea	11.1–13.9	8.8 TL		65	
tapirina			1.00		1.34
(Crawford 1984)	12.7–17.7	8–10 TL[f]		44–53	

[a] Standard length (SL) estimated from mass (dry weight) at first weekly census after metamorphosis by the length–weight equation of Laurence (1979).

[b] Approximated by Laurence (1975) based on growth rate during early larval life.

[c] Average of eight populations from table 1 of Policansky (1982).

[d] Total length (TL) at stage H of Seikai et al. (1986).

[e] Age at stage 1 of Seikai et al. (1986).

[f] Estimated from fig. 1 of Crawford (1984).

focus on metamorphosis but did provide data about metamorphosis. Length at metamorphosis was less variable than age at metamorphosis across environments (Table 3). Length and age at metamorphosis were negatively related across the two temperature environments; cooler temperatures resulted in small sizes at metamorphosis and protracted larval periods. Converting Laurence's values of length and age at metamorphosis to growth and developmental rates for the entire larval period reveals a strong positive relationship between these rates and between each rate and temperature.

As part of a larger study (discussed above), Policansky (1982) raised starry flounder in the laboratory at two temperatures. Length at metamorphosis decreased and age at metamorphosis increased with decreasing temperatures. Length was less plastic than age at metamorphosis (Table 3). Growth and developmental rates were positively correlated and increased with temperature.

Seikai et al. (1986) grew Japanese flounder (*Palalichthys olivaceus*) in the laboratory at approximately 13, 16, and 19°C. Length at metamorphosis was less variable than age at metamorphosis across temperatures (Table 3). Their results agree with Laurence's and Policansky's in that growth and developmental rates were positively correlated with each other and with temperature. Unlike Laurence's and Policansky's results, length at metamorphosis increased with decreasing temperature, resulting in a positive relationship across environments between length and age at metamorphosis.

Crawford (1984) reared each of two species of Tasmanian flounders in the laboratory at high and low temperature regimes. Larvae of *Ammontretis rostratus*, reared in two different years, were slightly longer and older at metamorphosis when reared at cooler temperatures (Table 3). The temperature regimes varied widely in Crawford's rearings, and as a further caveat, these two temperature regimes were confounded with the year in which larvae were reared. Larvae of *Rhombosolea tapirina* were affected less in their lengths at metamorphosis than their ages at metamorphosis in populations reared simultaneously at two temperature regimes (Table 3). The high and low temperature regimes were again variable. Length at metamorphosis was similar between the two temperature regimes but age at metamorphosis increased at the cooler temperatures. For both species, population averages for length at metamorphosis were not clearly related to age at metamorphosis. Growth and developmental rates across temperatures, however, were positively related to each other and with temperature.

Laurence (1977) assessed the effect of food level on growth rate through the larval period of winter flounder. Although the effect of food level on age at metamorphosis is not reported, we conclude from the fitted growth curves that mass at metamorphosis decreased with decreasing prey abundance. Mass at 7 wk post-yolk-sac absorption for prey levels of 3, 1, and $0.5 \cdot mL^{-1}$ was 539, 542, and 279 μg dry weight, respectively. This age was declared by Laurence (1975) as time to metamorphosis for winter flounder reared at 8°C and 2 $prey \cdot mL^{-1}$. Converting these values to lengths by the equation of Laurence (1979) gives 7.17, 7.17, and 6.24 mm SL at 7 wk. All tanks were stocked with the same number of hatchlings but the number of survivors, corrected for destructive sampling, varied from 171 to 19 to 13 across descending food levels. The small increase in mass and SL between the 0.5 prey·mL^{-1} and the higher prey density tanks (approximately 194 and 114%, respectively) may reflect compensatory growth at the lower prey densities. If developmental times were at all retarded at lower prey densities, then the differences in sizes at metamorphosis between the lowest and other prey densities would be even less.

Houde and colleagues have reared the lined sole past metamorphosis and have measured the growth response of larvae to varied food and crowding levels (Houde et al. 1970; Houde 1977; Houde and Schekter 1981). Early work (Houde et al. 1970) revealed a 16-d duration of the larval period. In subsequent rearings, Houde (1977) found both length and mean mass at 16 d to decrease significantly with decreasing food levels and to decrease, although not significantly, with increased crowding. Density-dependent mortality and compensatory growth may have reduced the effect of crowding. Houde and Schekter (1981) listed bidaily length at age data for soles reared under varying food regimes. By applying the values of Houde et al. (1970) for size and age at onset of metamorphosis (3.2 mm SL and 10 d, respectively), we estimate that reduced food levels extended the age at onset of metamorphosis (age at 3.2 mm SL) from 8.5 to 12 to 14 d at 1000, 100, and 50 prey·mL^{-1}, respectively. The length at onset of metamorphosis (length at age 10 d) declined from 3.5 to 2.9 to 2.8 mm SL for the above food levels. By framing the metamorphosis window in this crude way, we conclude that reducing food supply results in metamorphosis at smaller sizes, older ages, or both.

Riley (1966) provided an intriguing speculation on the relative plasticity of size and age at metamorphosis in laboratory populations of European plaice. When plaice larvae were subjected to an array of six food levels, Riley found that the final length of the postmetamorphic fish was "unnaturally small" but that the fish from the lowest two food levels metamorphosed only 63 h later than those in the highest two food levels. This difference was equivalent to a 6.4% retardation of the period from first feeding to metamorphosis. Riley concluded that metamorphosis is not controlled by size but by "a mechanism dependent mainly on temperature and age" (temperature increased throughout the course of Riley's rearings). Lengths at metamorphosis were not reported, but final lengths (day 84, fig. 8 of Riley), taken at approximately 14 d postmetamorphosis, show a 29% change from the poorest two to the richest two food environments. Riley noted that most of the variance in final lengths of fish in the highest two food levels was generated during the last 2 wk of rearing which approximates the duration of time since metamorphosis in those tanks. We recalculated the increase in the larval period duration (from fig. 9) and compared final lengths (from fig. 8) for the lower four food levels (Riley's C through F food densities which span a sixfold range). Larval duration was extended by 2.1% in the poorest food environment, while average final lengths increased by 22% between the poorest and richest food levels. This is a conservative corroboration of Riley's conclusion on the restricted variation in age relative to size at metamorphosis. This suggests a difference from the patterns of relative variation described above for other flatfish and should be investigated further.

Significance of Variation in Metamorphic Characters

A general picture emerges from the studies to date. Length is less variable than age at metamorphosis within populations under constant conditions and in at least one natural population. Furthermore, their relative magnitudes appear consistent in all data examined (Tables 1, 2). Growth and developmental rates are positively correlated, and as this correlation weakens, size at metamorphosis increases with the duration of the larval period. Across-environment comparisons show size at metamorphosis to be less variable than age at metamorphosis, and in all cases, growth and developmental rate decrease (age at metamorphosis increases) with decreasing temperature (Table 3). Decreased food and increased crowding probably have the same effect. Needed are more studies, in both constant and manipulated environments, that report the size and age characteristics of *individual* fish at the time of transition. Studies involving environmental manipulation must include a minimum of two replicated populations per environment (Cochran and Cox 1957).

The quantity of phenotypic variance in a trait may reflect the correlation of that trait with fitness. Collins (1980) argued that less variable traits have a higher correlation with fitness; however, the partitioning of the observed phenotypic variance into its environmental and genetic sources is a prerequisite for any inference about the importance of a trait (Fisher 1930; Falconer 1981). It is generally true that lower levels of heritable variation reflect a tighter association of that character with fitness (Mousseau and Roff 1987). Our analysis of the parental influence on length and age at metamorphosis of winter flounder shows that the variation in length at metamorphosis, although roughly half the magnitude of variation in age at metamorphosis, was significantly influenced by parentage. Most of the variation in age at metamorphosis, however, was attributable to within populations variance and is, therefore, of nonheritable genetic or environmental origin (R. C. Chambers and W. C. Leggett, unpubl. data). The amount of the variance in these phenotypic characters, unlike most published values, cannot be attributable to manipulated environmental factors, such as temperature, food level, or crowding, because all of our rearing tanks were treated alike.

A phenotypic characterization of fish at metamorphosis has double utility for our understanding of the early life history of marine species. From this vantage we can first view backward in ontogeny. Size and age at metamorphosis provide a record of the individual up to this point in the life cycle, and by integrating larval growth and developmental rates, they measure an individual's performance and residence time in the larval period. Second, with a size–age characterization of individuals at metamorphosis, we can view forward into the juvenile period. For flatfish and many other species that shift to a demersal habitat in the postlarval period, the size at and timing of transition may be predictive of success in the new habitat. Variation in size at metamorphosis and the duration of the larval period, therefore, displays the potential expression of these characters. Quantifying and contrasting these distributions in homogeneous environments, as we report, is a first step in evaluating the consequences of varying environments on these characters.

Population Consequences of Variation in Size and Age at Metamorphosis

We expect rates of prey consumption, competition, predation, and mortality in general to vary with size in both larval and juvenile periods. Swimming speeds increase with sizes of larvae

(Ryland 1963; Hunter 1972), and these no doubt influence predator avoidance (Folkvord and Hunter 1986) and prey capture. The rate of consumption of prey (Laurence 1977; Houde and Schekter 1980) and, indeed, the ability to consume larger prey (Hunter 1981) increase with size of the larvae. A positive feedback, ontogenetic response wherein larger larvae get more food and thus get larger is likely to amplify the variance in size among larvae, regardless of the initial source of that variance. The limited evidence available, although from a variety of taxa, indicates that larvae with high growth and/or developmental rates become larger juveniles (Victor (1986) and Ochi (1986) for reef fish; Haley and Newkirk (1978) and Newkirk and Haley (1982) for oysters; Smith (1987) for frogs). For flatfish, a greater size at metamorphosis may yield a higher probability of survival as the fish switches from the three-dimensional pelagic environment to what is, effectively, a two-dimensional benthic habitat in which both exploitative and interference competition are more likely. Pearcy (1962b) indicated that for winter flounder the period shortly after metamorphosis is potentially a period of density-dependent growth. Mortality in the first few months of the juvenile period of European plaice is size specific and density dependent due, primarily, to predation by shrimp (van der Veer 1986; van der Veer and Bergman 1987). Peterman and Bradford (1987) concluded that density-dependent growth in English sole (*Parophrys vetulus*) occurs in their second season of juvenile growth (data on younger fish were not available). Our data show that increased size at metamorphosis is correlated with a longer larval period. The costs or advantages of specific sizes at metamorphosis have yet to be assessed, but there may not necessarily be a trade-off between size at metamorphosis and continued residence in the larval habitat. Early metamorphs, although relatively small, had higher growth during the larval period and this growth advantage continued into the early juvenile period (R. C. Chambers and W. C. Leggett, unpubl. data).

Age at metamorphosis is a key life history parameter for organisms with complex life cycles because it measures the duration of a life period. If mortality rates are life period specific (e.g. Smith 1985), then estimating the duration of a period is as crucial as estimating the rates that apply within the period. Interannual variability in the duration of the larval period at constant daily mortality rates has the potential to contribute significantly to the variation in survival to the juvenile period and, hence, recruitment to the fishery. For example, we applied an elementary geometric decay model (Goldberg 1958) to winter flounder survival through the larval period. We used a mortality estimate of $0.14 \cdot d^{-1}$ (Pearcy 1962a) and a between-environment difference in larval period duration of 49–80 d, as might be generated in an interannual or intraseasonal decrease in temperature of 3°C (Table 3). This difference in age at metamorphosis generated more than a 100-fold change in number of larvae surviving to metamorphosis, *ceteris paribus*.

The timing of metamorphosis may also quantitatively change the intra- and interspecific interactions due to their size specificity and the correlation of age and size at metamorphosis. Furthermore, qualitative changes in the guild of predators, prey, and competitors encountered in the juvenile habitat may vary with the timing of metamorphosis. Jeffries and Johnson (1974) and Jeffries and Terceiro (1985) argued that slight among-year temperature differences of 1°C were responsible for fluctuations in abundances of winter flounder in an estuary in Rhode Island. They described a scenario in which winter flounder metamorphose earlier in warmer years and are subjected to higher predation rates as they arrive at the sea floor.

Sinclair and Tremblay (1984) provided insight into the demographic link between the larval and adult periods. Their data from 27 different populations of Atlantic herring (*Clupea harengus harengus*) show that (1) the duration of the larval period (age at 40 mm length) varies among populations from 2.5 to 11 mo and (2) the spawning period, which is generally less than 1 mo in duration, is seasonally fixed and characteristic of the population. The timing of spawning, as argued by Sinclair and Tremblay, depends on the characteristics of the inferred larval drift and feeding area which, in turn, dictate the duration of the larval period. Herring populations associated with relatively food-rich areas, characteristic of stratified water columns, spawn in the spring and their offspring metamorphose before fall. Well-mixed waters are poorer for larval growth, and populations spawning there do so in autumn or winter. The offspring in these populations metamorphose in the following warm season. These combined findings strongly suggest that additional analyses of population-specific growth, developmental, and mortality rates, as well as fluctuations in abundances, are warranted and may further isolate the sources of interstock variation in the life histories of fishes.

Two points arise from our results and our survey of the literature on metamorphosis in marine fish. First, the patterns of variation and correlation in size and age at metamorphosis are known for only a few species. Any generalizations are necessarily conditional until more data are collected on these characters, but length at metamorphosis is consistently less variable than age at metamorphosis at approximately a 1:2 ratio of CVs within populations and is less plastic to environmental change. Furthermore, growth and developmental rates of individuals seem inextricably and positively related. The causes and maintenance of the intrapopulation variance in these characters observed by us, Policansky (1982), and Victor (1986) are still open questions. Second, the ecological consequences of variation in size at metamorphosis and the duration of the larval period are potentially pervasive, although as yet there have been few attempts to evaluate these consequences.

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Ammonium Transformations in Springwater within the Riparian Zone of a Small Woodland Stream

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Hill, A. R., and J. Warwick. 1987. Ammonium transformations in springwater within the riparian zone of a small woodland stream. Can. J. Fish. Aquat. Sci. 44: 1948–1956.

Field enrichments with ammonium were conducted for periods of 4–4.5 h on two springs in the riparian zone of a small woodland stream near Toronto, Ontario. Springwater lost much of the injected ammonium as it flowed over organic and sandy riparian substrates before entering the stream. Negligible nitrate release occurred during the ammonium enrichments. Laboratory experiments revealed that riparian substrates rapidly removed ammonium from enriched springwater. Sediments covered with aerated ammonium-enriched water showed a small nitrate-N increase of 9–12 $\mu g \cdot L^{-1}$ after 5 h. An absence of ammonium depletion in sterilized organic substrates and very small losses in low-temperature (1°C) incubations suggested that microbial activity rather than adsorption was responsible for ammonium uptake. The rapid loss of ammonium from springwater overlying acetylene-treated substrates, despite the complete inhibition of nitrification, indicated that microbial immobilization was mainly responsible for ammonium depletion. We suggest that ammonium transformations in spring-fed areas of overland flow within the riparian zone may contribute significantly to the regulation of nitrogen inputs to small woodland streams.

On a enrichi d'ammonium, pour une période de 4 à 4,5 h, deux sources de la zone riveraine d'un petit ruisseau sylvestre près de Toronto (Ontario). L'eau de source a perdu une grande partie de l'ammonium injecté en coulant sur les substrats organiques et le sable de la rive avant de s'incorporer au ruisseau. La libération de nitrate a été négligeable pendant l'enrichissement en ammonium. Les expériences de laboratoire ont révélé que les substrats riverains éliminent rapidement l'ammonium de l'eau de source enrichie. Des sédiments recouverts d'eau aérée, enrichie d'ammonium, présentent une faible augmentation de l'azote sous forme de nitrate de l'ordre de 9 à 12 $\mu g \cdot L^{-1}$ après 5 h. L'absence d'une diminution de l'ammonium dans les substrats organiques stérilisés et les très faibles pertes lors d'incubations à basse température (1°C) indiquent que l'activité microbienne, et non l'adsorption, est responsable de la captation de l'ammonium. La perte rapide d'ammonium dans l'eau de source recouvrant des substrats traités à l'acétylène, en dépit de la complète inhibition de la nitrification, indique que l'immobilisation microbienne est principalement responsable de la diminution de l'ammonium. Nous suggérons que la transformation de l'ammonium dans les zones de ruissellement alimentées par des sources aux abords des rives peut contribuer de façon significative à régulariser l'apport en azote des petits ruisseaux sylvestres.

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The role of riparian zones in modifying the chemistry of groundwater before it enters stream channels is not well documented. Recent studies indicated that forest riparian zones in lowland agricultural watersheds in the southeastern United States were effective in retaining several nutrients, particularly nitrates, which entered the riparian zone in groundwater from fertilized fields (Lowrance et al. 1984; Peterjohn and Correll 1984; Jacobs and Gilliam 1985). In contrast, Peterjohn and Correll (1984) found that concentrations of ammonium in groundwater increased during transport through the riparian zone.

The effect of the riparian zone on nutrient inputs to streams in undisturbed headwater drainage basins has not been investigated. Nutrient concentrations in groundwater entering the riparian zone from upland forest communities are likely to be low, and the effects of riparian zone transformation processes on nutrient transport may differ from those occurring in agricultural watersheds. Moreover, the few studies that deal with nutrient transformations in riparian areas have focused mainly on the chemistry of shallow groundwater. In headwater streams, groundwater often emerges as springs and seeps creating areas of diffuse saturated overland flow in the riparian zone. Hynes (1983) has suggested that the movement of water by different routes in the riparian zone may have an important influence on the supply of nutrients to streams. However, nutrient transformations in riparian springs have been neglected with the exception of a study by Rigler (1979) which showed that phosphorus was rapidly depleted in springwater which percolated over tree roots and a moss bed before entering the stream.

This study examines ammonium transformations in spring-fed areas of overland flow within the riparian zone of a forested headwater stream. Ammonium was selected for study because this element may influence rates of decomposition and algal production in streams. Several researchers have suggested that ammonium is taken up in preference to nitrate by aquatic bacteria, fungi, and algae (Thornton 1963; Brezonik 1972;

Stanley and Hobbie 1980; Zevenboom and Mur 1981). Riparian areas have a potential to either increase or decrease ammonium fluxes entering stream channels. Microbial mineralization of even a small fraction of the organic matter which is often abundant in riparian soils could release considrable quantities of ammonium into the near-surface groundwater as it approaches the stream. Conversely, a number of processes can act to deplete ammonium within the riparian zone. These processes include vegetation uptake, ammonium adsorption onto riparian substrates, and microbial transformations involving immobilization and nitrification.

We investigated nitrogen transformations by experimentally enriching riparian zone springs with ammonium. Laboratory experiments were also used to evaluate processes of nitrogen uptake and release from riparian substrates. We focused on interactions between substrates and spring-fed areas of overland flow because previous research indicated that microbial processes rather than vegetation uptake were mainly responsible for the regulation of nitrogen fluxes in riparian forests (Peterjohn and Correll 1984; Jacobs and Gilliam 1985).

Study Area

The study area is located in a small forested headwater drainage basin of 2 km^2 on the south-facing slope of the Oak Ridges moraine about 50 km northeast of Toronto, Ontario. The watershed is characterized by moderately to steeply sloping hummocky moraine dissected by dry valleys and is drained by a second-order stream and several perennial tributaries. Stream discharge varied between 40 and 50 $L \cdot s^{-1}$ and ammonium-N and nitrate-N concentrations were <10 and 40–100 $\mu g \cdot L^{-1}$, respectively, at the basin outlet during the summer of 1984.

Grey–Brown Luvisols formed on glacial sands, and gravels occur on the moraine slopes, whereas organic soils (Histosols), which vary in thickness from a few centimetres at the upland margin to 1.5 m near the streams, are found on the riparian area. About 60% of the watershed is forested with mature sugar maple –beech (*Acer saccharum – Fagus grandifolia*) and younger stands of white pine – birch (*Pinus strobus – Betula papyrifera*) on the upland slopes. A mature forest of hemlock (*Tsuga canadensis*) and cedar (*Thuja occidentalis*) occurs on the riparian area. The remainder of the watershed is occupied by an old-field community of grasses and forbs with scattered patches of trees.

A groundwater discharge zone at the base of the moraine slopes produces a saturated riparian area 20–100 m wide along the second-order stream. The water table intersects the ground surface at the upland margin of the riparian zone and then slopes towards the stream at a depth of 10–30 cm. Springs and seeps at the base of the moraine produce numerous small drainage lines which cross the riparian zone and contribute about 50% of the groundwater entering the stream (Dr. N. Roulet, Department of Geography, York University, pers. comm.). The term "drainage line" has been defined as a line of flow having some depth but not necessarily contained within banks formed by water erosion (McCaig 1983). The drainage lines in this study are usually less than 40 cm in width and have water depths of 2–15 cm and discharges of <1 $L \cdot s^{-1}$. Surface flow often passes into shallow pipes and cavities formed by dead tree roots and partially uprooted trees. Subsurface groundwater moves upward to the water table throughout the riparian area. The entry of this groundwater at locations where the surface drainage lines intersect the shallow water table produces a flow increase of 50–100% in individual drainage lines within the riparian zone. Groundwater also reaches the stream by subsurface routes as bank and bed seepage.

Enrichment experiments were done on two representative drainage lines within the riparian zone of the second-order stream. Drainage line 1 flows for about 50 m in a series of alternating surface and pipe segments before dividing into two separate flow lines which follow an erratic course mainly in pipes before entering the stream at outlets 25 m apart. Drainage line 2 contains no pipe sections and flows for 105 m through the riparian forest to the stream. Water in the drainage lines flows over various substrates which include organic-rich sediments consisting of partly decomposed plant litter and eroded organic soil, accumulations of woody debris, and sandy sediments derived from glacial deposits exposed near the upland margin of the riparian zone. Properties of the substrates are shown in Table 1.

The drainage lines are heavily shaded and aquatic plants are generally absent. Water temperature increased from 9°C in May to 10–13°C in August and pH varied between 7.6 and 8.0. During the enrichment experiments, discharge in drainage line 1 increased from 0.5–0.7 $L \cdot s^{-1}$ at 50 m downstream to 0.8–1.0 $L \cdot s^{-1}$ for the combined discharge of the two outlets entering the stream. Discharge in drainage line 2 was usually 0.1 – 0.2 $L \cdot s^{-1}$ at 15 m below the source and 0.3–0.5 $L \cdot s^{-1}$ at 80 m. Background concentratons of ammonium-N in the drainage lines were <10 $\mu g \cdot L^{-1}$. In contrast, subsurface groundwater inputs to the drainage lines contained ammonium-N concentrations of 30–50 $\mu g \cdot L^{-1}$.

Materials and Methods

Field Enrichment Procedures

Ammonium enrichments were conducted for periods of 4–4.5 h on May 15, June 26, and August 23 in drainage line 1 and on May 17, June 28, and August 27 in drainage line 2. The enrichment solution was prepared at the study sites by adding ammonium as NH_4Cl to water from the drainage lines in a 22-L carboy. Sodium chloride, mixed with the ammonium, was used as an inert tracer. The solution was dripped through a flow regulated tube at 1 $mL \cdot s^{-1}$ into each drainage line within a few metres of the spring source at a point where rapid mixing occurred.

Water samples were collected from the drainage lines before the experiment began and at intervals of 20 min until 1 h after enrichment had ended. Sample stations on drainage line 1 were located at 50 m below the enrichment point and at 96 and 101 m downstream on the west and east branch, respectively, where these entered the second-order stream. Samples were taken from drainage line 2 at 15, 60, and 105 m downstream. Ammonium, nitrate, and chloride concentrations were determined by standard techniques (Technicon 1977, 1978; Environment Canada 1979) using a Technicon AutoAnalyzer. Ammonium transformations in the drainage lines were evaluated by comparing measured concentrations of ammonium with concentrations predicted from the chloride tracer using the formula of Richey et al. (1985).

Laboratory Experiments

Interactions between riparian substrates and ammonium-enriched drainage line water were examined in the laboratory to establish that ammonium transformations observed in the field

TABLE 1. Mean chemical properties ± SD ($n = 7$) of 0–1 cm depth drainage line sediments.

Substrate	NH_4–N ($\mu g \cdot g^{-1}$)	NO_3–N ($\mu g \cdot g^{-1}$)	Organic C (%)	Total N (%)	C:N ratio	pH
Organic sediment	3.4±1.7	0.5±0.6	12.7±7.3	0.61±0.64	20.8	7.3±0.4
Sandy sediment	1.5±0.9	0.1±0.1	1.3±0.7	0.06±0.03	21.7	7.5±0.2

enrichment experiments could be caused by sediment–water exchanges. Laboratory experiments were also designed to assess the importance of three mechanisms associated with riparian substrates which could deplete ammonium from the drainage lines. These mechanisms are ammonium adsorption onto sediments, microbial immobilization, and nitrification.

Ammonium uptake and nitrification

Ammonium uptake and nitrification in drainage line substrates were evaluated using surface samples of sandy and organic-rich sediment. Approximately 20 g of fresh sediment was placed in flasks with 200 mL of drainage line water amended with 500 $\mu g\ NH_4\text{-N} \cdot L^{-1}$. One set of these sediment–water systems was shaken on a reciprocal shaker at 13°C. The use of vigorously mixed systems provides an estimate of maximum rates of sediment–water exchange (Lee 1970). A second set of samples was incubated under aerated conditions at 13°C. This temperature was used because water temperatures do not exceed 13°C during the summer in the riparian forest. The bubbling of air into the solutions also created some turbulence and kept the liquid aerated in an attempt to reproduce drainage line conditions.

Ammonium adsorption versus biological uptake

This experiment was designed to separate adsorption from biological uptake of ammonium in the drainage line substrates. Surface organic sediments from three sites were mixed to form a composite sample. Approximately 20 g of this mixed sediment was placed in flasks with 150 mL of drainage line water amended with 500 $\mu g\ NH_4\text{-N} \cdot L^{-1}$. One set of samples was incubated without further treatment, a second set of the sediments was autoclaved, a third had 0.2 mL of chloroform added to each flask, and a fourth set was incubated at 1°C. The flasks were shaken and, with the exception of the low-temperature treatment, were kept at 21°C. Water from three replicates of each treament was filtered through a 0.45-μm membrane filter for analysis of ammonium and nitrate at four intervals during the 48-h incubation.

Nitrification versus microbial immobilization

Biological utilization of ammonium in drainage line substrates may involve nitrification and microbial immobilization of ammonium. The role of nitrification in ammonium uptake was evaluated by examining ammonium consumption rates in control and acetylene-treated sediments. Acetylene completely inhibits the microbial oxidation of ammonium to nitrite (Walter et al. 1979). Sediment samples were collected from 10 sites. Approximately 20 g of fresh sediment and 200 mL of 500 μg $NH_4\text{-N} \cdot L^{-1}$ solution were placed in flasks. Acetylene was added to bring the headspace concentration to 10% (v/v) in one set of samples; a second set of the sediments was incubated without further treatment. The flasks were shaken at room temperature (21°C) and solution samples were collected after 5, 24, and 48 h.

Sediment analysis

Ammonium and nitrate in fresh drainage line sediments were extracted with 2 *M* KCl followed by automated analysis using the procedures outlined for water samples. Total nitrogen was analyzed using a Technicon BD-40 block digester followed by determination of the resulting ammonia on the AutoAnalyzer (Schuman et al. 1973). Substrate pH was measured with a glass electrode on a 1:2 sediment and water mixture and organic carbon was estimated by the Walkley–Black method.

Results

Field Enrichments

Data collected during the ammonium enrichment experiments are shown for four dates in Fig. 1 and 2. Data obtained on three other dates were similar and are therefore not presented. During the enrichments, ammonium and chloride concentrations below the injection point were 60–140 and 12–30 times greater than measured background levels in the drainage lines. However, the dilution effect of considerable groundwater inputs within the riparian zone produced a rapid downstream decrease in element concentrations (Fig. 1 and 2). The leading edge of the injected ammonium in the drainage lines travelled through the riparian zone in 30–40 min. Minor fluctuations in element concentration throughout the enrichment period result from an erratic supply of the solution from the carboy.

There was a substantial loss of ammonium from the water column in both drainage lines on all observation dates. About 20–25% of the injected ammonium was removed within 50 and 15 m in drainage lines 1 and 2, respectively (Fig. 1 and 2). In drainage line 2, almost all the ammonium was depleted on some dates between the injection point and the drainage line outlet to the second-order stream. Observed ammonium concentrations declined rapidly towards background levels within 1 h after enrichment ended. Nitrate concentrations which were monitored during the ammonium enrichments showed a small increase of 5–15 $\mu g\ N \cdot L^{-1}$ above background levels at sampling sites on drainage line 2 (Fig. 3). However, with the exception of August 23, nitrate concentrations in drainage line 1 remained relatively constant during the enrichments.

Laboratory Experiments

Organic sediments shaken at 13°C showed a rapid loss of ammonium from the water column throughout the 48-h incubation (Table 2). The sandy sediment–water slurries exhibited a more gradual decrease in ammonium. Considerable nitrate release occurred within 5 h in the shaken sediments (Table 2). Nitrate accumulation continued throughout the incubation and was particularly rapid in flasks containing organic sediments. Nitrite concentrations ranged from undetectable to only about 10 $\mu g\ N \cdot L^{-1}$. Despite the small quantity of ammonium

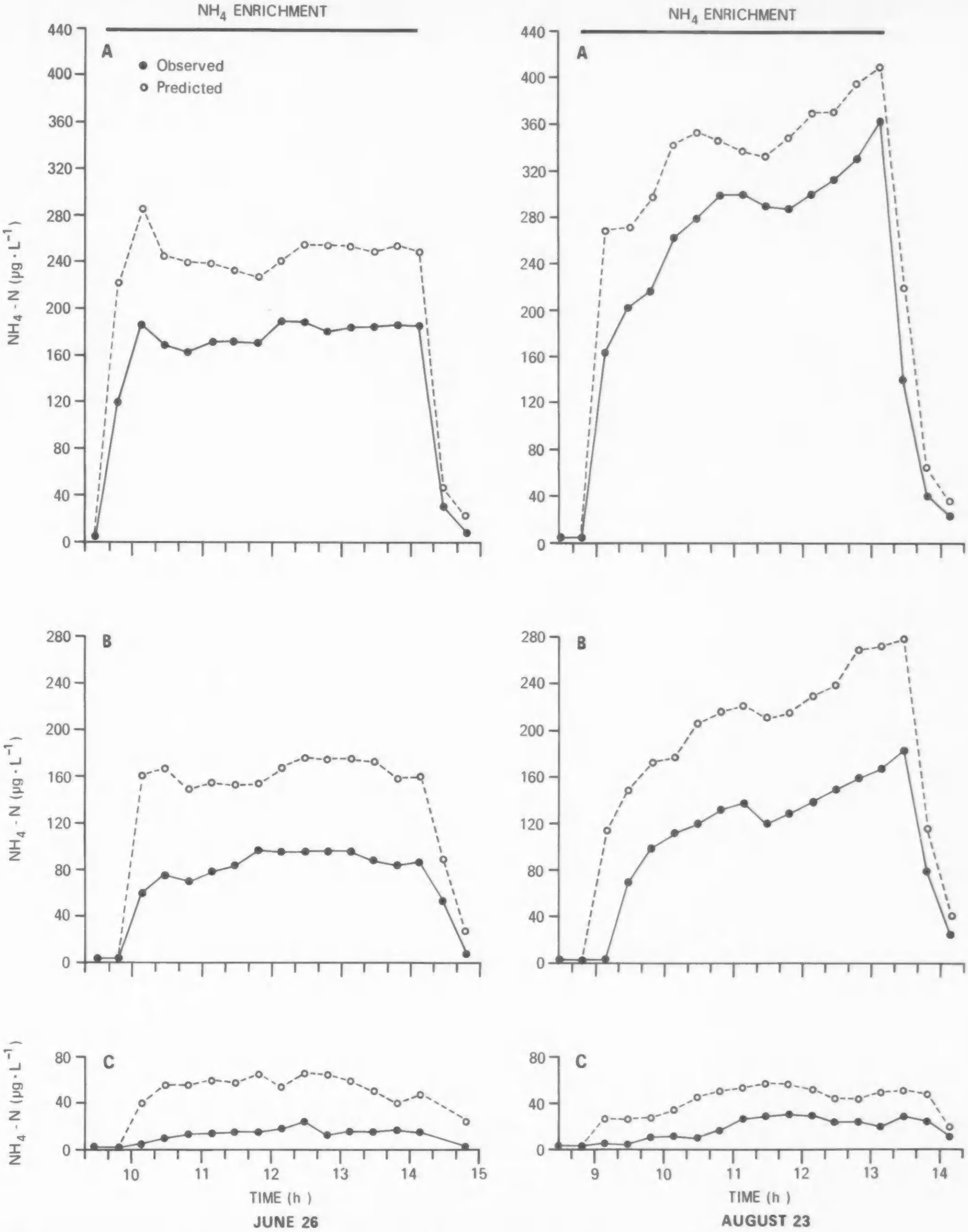

FIG. 1. Observed and predicted concentrations of NH$_4$-N in drainage line 1. (A) 50 m downstream; (B) west branch, 96 m downstream; (C) east branch, 101 m downstream. Predicted concentrations were estimated from observed chloride concentrations.

remaining after 24 h in the organic sediment slurries, nitrate production continued between 24 and 48 h, indicating that organic nitrogen mineralization and concurrent nitrification was occurring in these flasks.

Average ammonium concentrations in the water columns of the aerated sediments showed a decrease of 68–77 µg N·L^{-1} after 5 h and continued to decline slowly throughout the experiment (Table 2). In contrast with the sediment slurries, average rates of ammonium loss were similar for both sediment types during the initial 24 h of the aerated treatment. Nitrate

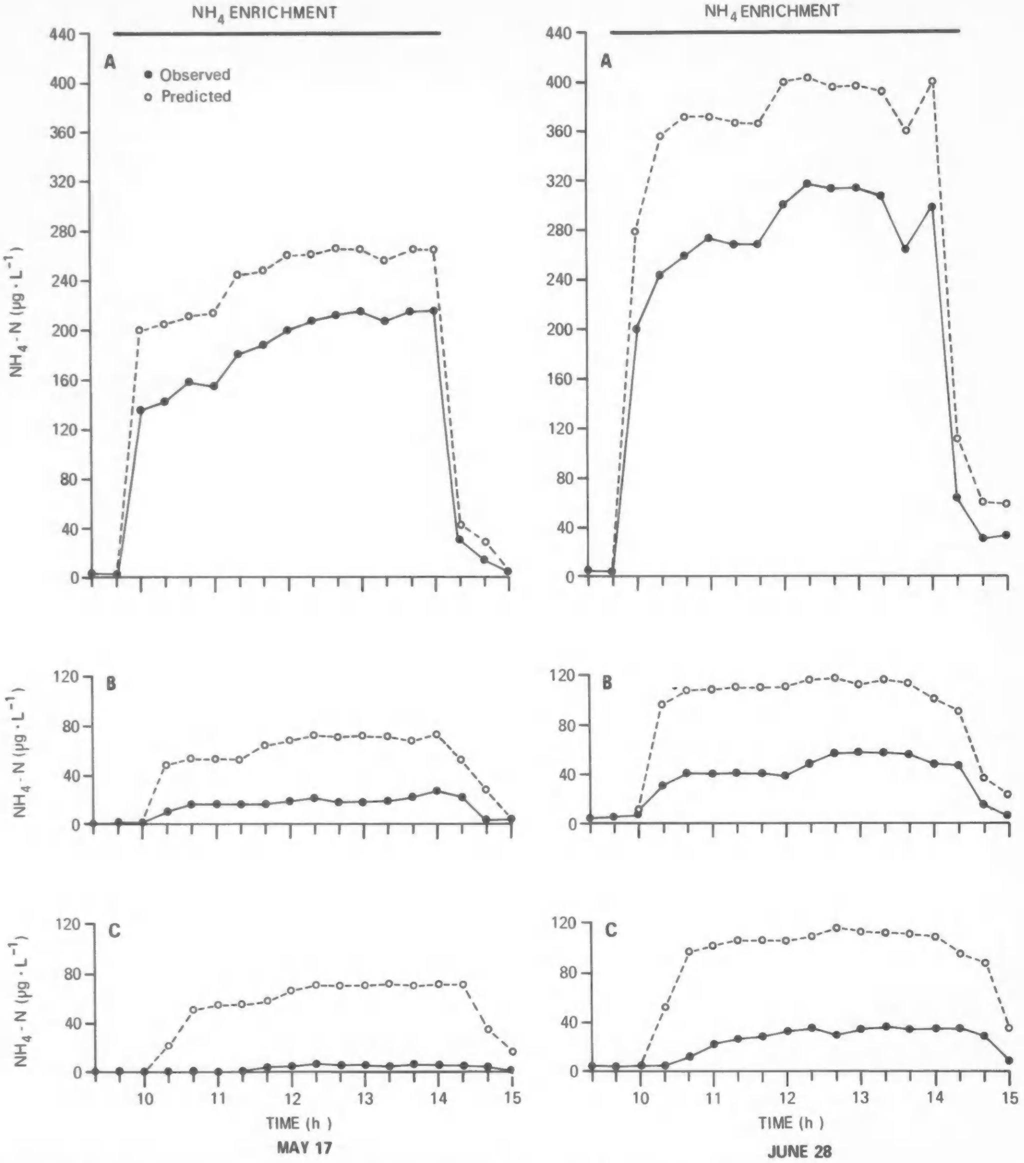

FIG. 2. Observed and predicted concentrations of NH$_4$-N in drainage line 2. (A) 15 m downstream; (B) 60 m downstream; (C) 105 m downstream.

accumulation was less rapid under aerated conditions than in the shaken sediments.

Ammonium loss did not occur in autoclaved and chloroform-amended treatments of organic sediment samples (Fig. 4). The initial increase of ammonium in autoclaved samples may result from alteration of organic nitrogen and ammonium release caused by elevated temperature and pressure (Eno and Poponoe 1964; Stanford and Demar 1969). However, after this initial increase there was no further change in ammonium during the 48-h incubation. The low-temperature treatment exhibited reduced ammonium loss and smaller amounts of nitrate release in comparison with unsterilized sediments shaken at 21°C. Nitrate did not accumulate in the autoclaved and chloroform-amended treatments.

Nitrification was inhibited in acetylene-treated sediments, whereas considerable quantities of nitrate accumulated in untreated sediments after 48 h (Table 3). Ammonium in water overlying acetylene-amended organic sediments disappeared rapidly despite the complete inhibition of nitrification. Sandy sediments treated with acetylene also showed ammonium uptake during the initial 5 h of the incubation. Although ammonium depletion in acetylene-amended sediments was

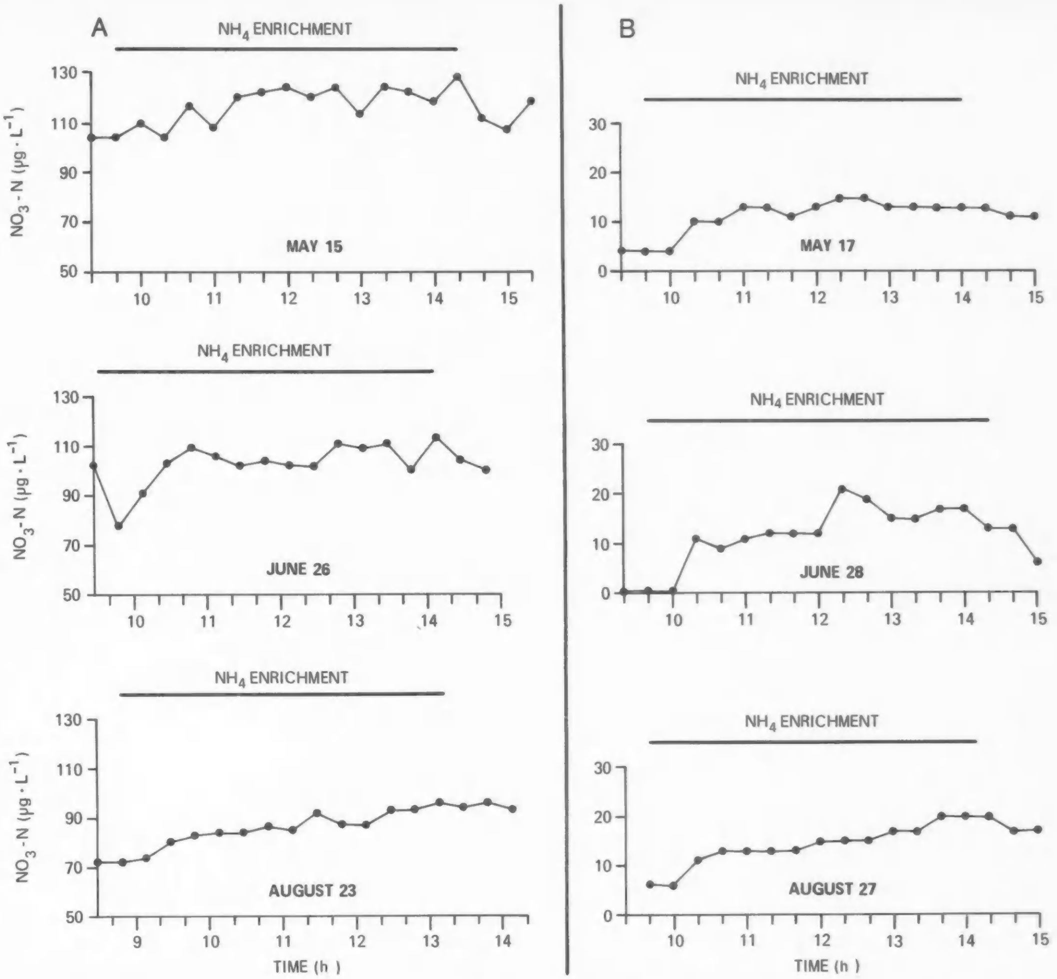

FIG. 3. Changes in NO_3-N downstream following addition of NH_4-N to the drainage lines. (A) Drainage line 1 west branch, 96 m downstream; (B) drainage line 2, 60 m downstream.

TABLE 2. Ammonium–N and nitrate–N concentrations ($\mu g \cdot L^{-1}$) in ammonium-enriched water incubated with drainage line sediments in the laboratory. Values are means ± SE ($n = 5$).

	5 h		24 h		48 h	
Substrate	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3
Shaken at 13°C with NH_4 enrichment[a]						
Organic sediment	256±77	149±18	76±24	377±66	45±37	635±128
Sandy sediment	389±19	72±8	346±23	127±24	319±14	202±39
Aerated at 13°C with NH_4 enrichment						
Organic sediment	423±11	47±3	367±9	100±13	284±20	192±46
Sandy sediment	432±13	44±2	385±12	74±13	359±16	117±24

[a] Initial solution 500 μg NH_4–$N \cdot L^{-1}$ and 33 μg NO_3–$N \cdot L^{-1}$.

TABLE 3. Ammonium–N depletion and nitrate–N release ($\mu g \cdot L^{-1}$) in ammonium-enriched water incubated with acetylene-amended and untreated drainage line sediments at 21°C. Values are means ± SE ($n = 5$).

	5 h		24 h		48 h	
Treatment	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3
Organic sediment with NH_4-enriched water[a]						
With C_2H_2	209±31	38±1	47±36	36±3	12±4	36±3
Without C_2H_2	140±33	151±20	12±8	328±61	7±3	527±98
Sandy sediment with NH_4-enriched water[a]						
With C_2H_2	389±17	38±1	424±47	43±1	496±86	45±2
Without C_2H_2	329±34	77±5	385±39	149±11	460±95	232±20

[a] Initial solution 485 $\mu g\ NH_4\text{–}N \cdot L^{-1}$ and 30 $\mu g\ NO_3\text{–}N \cdot L^{-1}$.

lower than in untreated sediments after 5 h, little difference between treatments was evident during the remainder of the incubation.

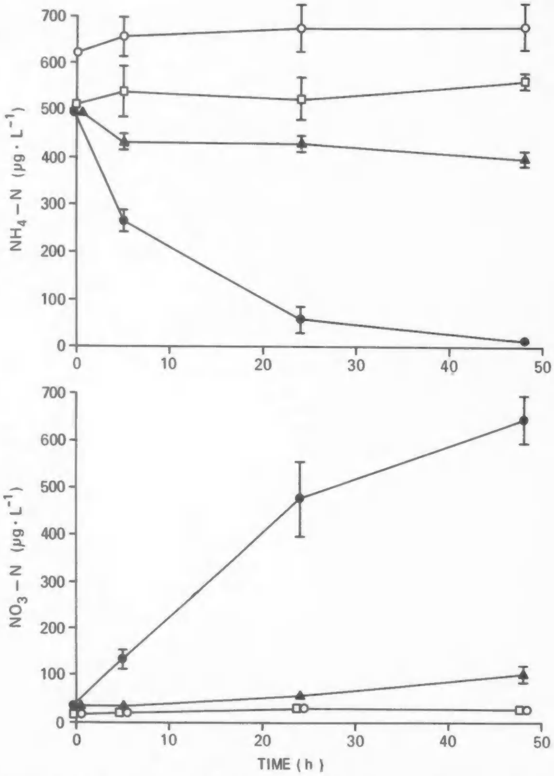

FIG. 4. Concentration of NH_4-N and NO_3-N over time in flasks containing ammonium-enriched water and organic drainage line sediment. Flasks were shaken at 21°C without further treatment (●), after autoclaving (○), with 0.2 mL of chloroform (□), and at 1°C (▲). All values are expressed as means ± SE ($n = 3$ replicates per mean). Points without error bars indicate SE smaller than the symbol itself.

Discussion

The field enrichment experiments indicate that considerable ammonium depletion can occur in spring-fed drainage lines. A comparison of predicted and observed ammonium concentrations at the drainage line outlets suggests that 40–60 and 80–95% of the ammonium was removed in drainage lines 1 and 2, respectively, during transport through a 100-m-wide zone of riparian forest. As a consequence of differences in discharge between the drainage lines, about 4–6 and 1–1.5 g of ammonium-N was injected into drainage lines 1 and 2, respectively, to achieve similar initial ammonium concentrations. Consequently, although drainage line 1 displayed a lower ammonium removal efficiency, the quantity of ammonium depleted was about 2.5 times greater than in drainage line 2.

The results of the ammonium uptake and nitrification experiment show that drainage line substrates have the ability to remove ammonium from the water column. The high rates of ammonium depletion observed in the sediment slurries are probably considerably greater than in situ rates. However, sediments incubated under aeration at 13°C to stimulate drainage line conditions more closely exhibited ammonium loss from the water column throughout the 48-h incubation. Both sediment types displayed a 14–15% decline in ammonium concentration in the water column after 5 h under aerated conditions (Table 2). These losses are of the same order of magnitude as the ammonium removal observed during field enrichments in the upper reaches of the drainage lines. These laboratory data suggest that uptake by the sediment by one means or another is a major mechanism of ammonium depletion in the riparian forest drainage lines.

The field enrichment experiments showed little evidence that ammonium depletion is linked to an increase in nitrate. However, sediment–water slurries incubated in the laboratory at 13°C exhibited a rapid accumulation of nitrate after 5 h, suggesting that under vigorously mixed conditions these substrates have a considerable nitrification potential. The aerated sediments incubated at 13°C probably replicate in situ drainage line conditions more closely than the sediment slurries. Mean nitrate accumulations after 5 h in the aerated sediments were 9–12 $\mu g\ N \cdot L^{-1}$ above the initial nitrate concentration in the water columns. This small nitrate release is similar to those observed in drainage line 2 during the enrichment experiments. However, such a small increase would have been difficult to detect in drainage line 1 because of higher background nitrate concentration. It is unlikely that the low nitrate release was a result of concurrent denitrification in the drainage line substrates. Short-term incubations using the acetylene blockage technique indicate very low in situ denitrification potentials in these sediments (J. Warwick and A. R. Hill, unpubl. data).

The absence of ammonium uptake in sterilized organic sediments and the negligible ammonium loss in the low-temperature treatment suggest that microbial transformations rather than adsorption are responsible for the ammonium depletion in the riparian drainage lines. The rapid decline in ammonium concentrations towards background levels in the drainage lines within 1 h after cessation of the enrichments also indicates that ammonium depletion did not result from adsorption. Adsorption of exchangeable ammonium by sediments is a reversible process with rapid desorption occurring at low ammonium concentrations (Rosenfeld 1979).

Microbial transformations involving either oxidation of ammonium by nitrifying bacteria or assimilation of ammonium by microorganisms could explain the observed depletion of ammonium by drainage line substrates. The results presented here indicate that the latter hypothesis is most likely. The rapid ammonium depletion in acetylene-amended organic sediments despite the inhibition of nitrification together with the absence of significant differences in ammonium concentrations between acetylene-treated and untreated sediments indicates that microbial immobilization of ammonium is considerably more important than nitrification in sediment ammonium uptake.

Although comparable research has not been undertaken in riparian areas, previous studies of small headwater streams have demonstrated rapid ammonium uptake during field enrichment experiments (Newbold et al. 1983; Richey et al. 1985). The absence of any substantial increase in nitrate during the enrichments of the riparian drainage lines contrasts with the results of Richey et al. (1985). They measured a nitrate increase of approximately 50–100 $\mu g\ N \cdot L^{-1}$ above background levels during a 6- to 10-h enrichment. Newbold et al. (1983) reported a small nitrate increase of 10 $\mu g\ N \cdot L^{-1}$ in the first few weeks, and a much larger release of 60 $\mu g\ N \cdot L^{-1}$ at the end of a 70-d continuous enrichment of a woodland stream in Tennessee. Rates of nitrate production after 5 h in our laboratory experiments with sediments overlain with aerated ammonium-enriched water were equivalent to 1.8–2.7 $\mu g\ NO_3\text{-}N \cdot g$ organic matter $(OM)^{-1} \cdot h^{-1}$, whereas Richey et al. (1985) reported rates of 3.5–7.8 $\mu g\ NO_3\text{-}N \cdot g\ OM^{-1} \cdot h^{-1}$ for organic stream substrates incubated under similar laboratory conditions. These results suggest that nitrifying activity in the riparian drainage lines is at the low end of the range measured in small forested first-and second-order stream channels.

Several characteristics of drainage line substrates may reduce the potential for autotrophic nitrification. Nitrifier activity may be limited by the supply of available ammonium. Nitrogen is often immobilized at a sediment C:N ratio of >20–25 (Alexander 1977; Vitousek 1981), although organic chemical composition and site characteristics may also affect the extent of nitrogen uptake (Berg and Staaf 1981). The mean C:N ratio of the drainage line sediments is about 21:1 (Table 1), and the laboratory experiments indicate that immobilization is a major mechanism of ammonium uptake. Consequently, strong competition with heterotrophic microorganisms may limit the ammonium available for nitrifiers. Nitrification may also be reduced by the low groundwater temperatures of the riparian zone which do not exceed 13°C in summer. Jones and Hood (1980) found that rates of ammonium oxidation by a nitrifying bacterium isolated from a freshwater wetland were about two times greater at 20°C than at 10°C.

Although nitrification occurred in riparian substrates, our results suggest that microbial immobilization was an important mechanism producing ammonium depletion during field enrichments of the drainage lines. Immobilization may also act as a sink for ammonium in subsurface groundwater inputs to the drainage lines within the riparian zone. Although flow in the drainage lines is augmented 50–100% by groundwater which has an ammonium concentration of 30–50 $\mu g\ N \cdot L^{-1}$, sampling at 1-to 2-wk intervals in the summer of 1984 revealed low ammonium concentrations of <10 $\mu g\ N \cdot L^{-1}$ in the drainage lines between the spring source and the outlets to the second-order stream.

It is possible that ammonium uptake by the microbial community may only produce temporary storage of nitrogen in the riparian zone. Ammonium immobilized in drainage line substrates may subsequently be mineralized or exported from the riparian zone as organic nitrogen. Further research involving the construction of a nitrogen budget for the riparian zone is being initiated to answer this question.

Although recent studies of nutrient fluxes in riparian areas have focused on the chemistry of subsurface groundwater, our data indicate that spring-fed areas of overland flow may also have a considerable potential to regulate nutrient fluxes. An increased understanding of the effects of forest riparian zones on the chemistry of small headwater streams will require greater knowledge of how groundwater chemistry is altered along different flow paths in the riparian zone.

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Evaluation of Techniques for Fixing, Preserving, and Measuring Salmon Eggs

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Fleming, I. A., and S. Ng. 1987. Evaluation of techniques for fixing, preserving, and measuring salmon eggs. Can. J. Fish. Aquat. Sci. 44: 1957–1962.

In a comparison of Stockard's solution with 5% buffered formalin for the preservation of coho (*Oncorhynchus kisutch*) and chum salmon (*O. keta*) eggs, formalin caused a lesser alteration in weight regardless of whether the eggs were preserved before or after waterhardening or fertilization. Coho and chum salmon eggs responded similarly to the various treatments. Time before preservation (3, 7, and 24 h) and fertilization had little effect on the final size of eggs. Waterhardening, in which the egg was placed in standing water, caused a 23% increase in fresh egg weight. Preservation in Stockard's solution, especially after waterhardening, increased egg weight by 48% and was least consistent in its effect. By contrast, 5% buffered formalin added to fresh eggs caused no significant alteration in weight, even after 5 mo of preservation. The latter was therefore the most suitable technique of preserving egg size. The use of egg weight as a measure of egg size was more accurate and consistent than linear measures of diameter. Finally, some empirical relationships for correcting published data on preserved egg weight are presented.

En comparant la solution de Stockard et une solution de formaline à 5 % tamponnée pour la conservation des oeufs de saumon coho (*Oncorhynchus kisutch*) et de saumon kéta (*O. keta*), on a observé une modification moins importante du poids avec la formaline, que les oeufs aient été traités avant ou après durcissement dans l'eau ou fécondation. Les oeufs de saumon coho et kéta se sont comportés de la même façon face aux divers traitements. La période précédant le traitement de conservation (3, 7 et 24 h) et la fécondation a peu d'effet sur la taille finale des oeufs. Le durcissement dans l'eau, qui s'opère en laissant séjourner les oeufs dans de l'eau stagnante, provoque une augmentation de 23 % du poids des oeufs frais. La conservation dans une solution de Stockard, surtout après durcissement dans l'eau, augmente le poids des oeufs de 48 % et l'effet est moins constant. Par contre, l'addition de formaline à 5 % tamponnée à des oeufs frais ne modifie pas de façon significative le poids des oeufs, même après 5 mo de conservation. Cette dernière technique est donc la meilleure pour conserver la taille des oeufs. Le poids est plus précis et plus constant que la mesure du diamètre pour évaluer la taille des oeufs. Enfin, on présente certaines équations empiriques pour corriger les données publiées sur le poids des oeufs traités à des fins de conservation.

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Knowledge of egg size is important for salmon management and life history research. The size of eggs can influence estimates of fecundity, calculations of the optimum time to initiate feeding (Rombough 1985), identification of salmon stocks (Craig 1985), and analysis of life history (Fleming and Gross 1988).

However, methods for studying salmon egg size are not consistent. Eggs have been collected at various states of gonad maturity (e.g. Stauffer 1976) and preserved and measured with various techniques. Eggs are often waterhardened and/or fertilized prior to preservation. During waterhardening, water is rapidly absorbed across the membrane causing the egg to become turgid (see Potts and Rudy 1969; Laale 1980). The egg may then be preserved in either (1) Stockard's solution, a mixture of formaldehyde, glacial acetic acid, glycerin, and distilled water (e.g. Drucker 1972), or (2) a low-concentration formalin solution (e.g. Morrison et al. 1985; Beacham and Murray 1986). These formaldehyde-based solutions generally serve the dual functions of fixation (i.e. rapid killing and stabilization of tissues) and preservation (i.e. maintenance of fixed tissues) of fish eggs because alcohol, a common preservative for other tissues, causes substantial dehydration and shrinkage (Snyder 1983). Here the term "preservation" will be used to include fixation.

The problems associated with preservation and measurement of fish eggs have only been addressed superficially in the literature (see Heming and Preston 1981; Snyder 1983; Markle and Frost 1985). This paper presents an experimental comparison of the effects of popular preservation techniques and measurement methods of salmon eggs. The purpose was to determine which preservation methods minimize alteration of natural egg size. In addition, we develop empirical relationships allowing comparison of egg sizes reported in the literature.

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Methods and Materials

Egg Collection and Preservation

Eggs from six reproductively mature ("ripe") female coho salmon (*Oncorhynchus kisutch*) of the November 1985 brood stock of the Capilano River Hatchery, North Vancouver, B.C., were stripped, placed in sealed plastic containers, and held at approximately 18°C until manipulation. Female postorbital–hypural length (i.e. distance from the posterior margin of the orbit to the end of the hypural plate) was recorded. Within 2 h, 90 ripe eggs per female were individually weighed to the nearest 0.1 mg wet weight, grouped into random lots of 5, and placed into 1 of 18 preservation treatments. The treatments consisted of combinations of egg state (fresh, waterhardened, or fertilized–waterhardened), time before preservation (3, 7, or 24 h), and preservative (Stockard's or 5% buffered formalin). Following the initial weighing of the eggs, fertilization and waterhardening were conducted. Eggs were fertilized for 5 min with a mixture of sperm from four males and then rinsed and waterhardened. Waterhardening, which is completed within 3 h (Potts and Rudy 1969), involved placing eggs in standing river water until they were to be preserved (3, 7, or 24 h). Waterhardened and fertilized–waterhardened eggs were reweighed immediately prior to preservation. Preservative solutions were (a) Stockard's solution (50 mL of formaldehyde, 40 mL of glacial acetic acid, 60 mL of glycerin, and 850 mL of distilled water; Velsen 1980) or (b) 5% buffered formalin (50 mL of formaldehyde, 4.0 g of sodium phosphate monobasic, and 6.5 g of sodium phosphate dibasic, made to 1 L with distilled water; Velsen 1980; Markle 1984).

Eggs from six reproductively mature female chum salmon (*O. keta*) of the Chehalis River Hatchery stock, Harrison Mills, B.C., were also stripped in November 1985 and held in sealed plastic containers at approximately 18°C until manipulation. Female postorbital–hypural length was recorded. Within 2 h, 40 ripe eggs per female were individually weighed, grouped into random lots of 10, and placed into one of four treatments: (1) 5% buffered formalin, (2) Stockard's solution, (3) waterhardened and 5% buffered formalin, and (4) waterhardened and Stockard's solution. Fertilization had no significant effect on coho egg weight (see Results) and was, therefore, not performed with chum salmon. Eggs in treatments 3 and 4 were waterhardened immediately following initial weighing and all eggs were preserved 3 h after their last manipulation.

After 7 d of preservation, eggs were weighed to determine the extent of alteration of natural egg size. Random subsamples of coho and chum eggs from each treatment were also reweighed 5 mo later to determine if any further changes in egg size had occurred.

Egg diameters, both long and short axes, were measured to 0.04 mm using a dissecting microscope at a power of 180× for random subsamples of coho and chum eggs. For each species, four treatments were examined: (1) 5% buffered formalin (n = 15); (2) Stockard's solution (n = 15); (3) waterhardened and 5% buffered formalin (n = 20); and (4) waterhardened and Stockard's solution (n = 20). In addition, fresh egg diameters were also measured for the 5% buffered formalin treatment prior to preservation.

Measurement error was determined by repeated measures of weight and diameter for a random subsample of coho (n = 15) and chum salmon eggs (n = 15).

Statistical Analyses

Except for regression analysis of egg diameter on egg weight, all data were expressed as ratios of treated egg weight to initial fresh egg weight. The coho salmon data were examined by analysis of variance, with egg state (n = 3), time before preservation (n = 3), and preservative (n = 2) treated as fixed effects and individual fish (n = 6) treated as a random effect (see Table 1). Some preservation treatments caused one or two eggs per fish to burst, destroying the eggs. To balance the data set, we randomly selected three eggs per fish from each cell (n = 18). The full statistical model, including all first-, second-, and third-order interactions, was fitted to the ratio of preserved weight after 7 d to initial weight. Residual analysis revealed that the variance of the residuals increased as the ratio increased. Therefore, log-transformed data were used.

Coho were compared with chum using a mixed, nested model (fish nested in species). This analysis of variance had two egg states, one time before preservation (3 h), two preservatives, two species, six fish, and three randomly selected replicates per cell. Again, log-transformed data and the ratio of preserved to fresh weight were used.

Repeatability (i.e. coefficient of intraclass correlation; Sokal and Rohlf 1981) of individual female egg weight was calculated using a random subsample of five eggs per treatment (ignoring time before preservation) per fish. The coefficient is a measure of the correlation between weights of different eggs from the same female, thereby providing an indication of how many eggs need be measured to obtain a representative sample for each female.

Regression models were developed for egg diameter on weight. Tests of coincidence were conducted between regression lines for chum and coho salmon and among treatments using multiple regressions with dummy variables.

Results

There was little variation in fresh egg weight within females (coho: range of CV = 3.18–6.75%, n = 90 eggs per female; chum: range of CV = 1.90–6.09%, n = 40 eggs per female). However, fresh coho salmon eggs ($\bar{x}$ = 168.4 mg, SD = 35.8, n = 6 females) were smaller than fresh chum salmon eggs ($\bar{x}$ = 208.8 mg, SD = 9.2, n = 6 females; t = 2.68, P = 0.023), as were the females from which they came (coho: $\bar{x}$ = 547.5 mm postorbital–hypural body length, SD = 48.6; chum: $\bar{x}$ = 633.5 mm, SD = 21.2; t = 3.97, P = 0.003). For this small data set no significant correlation existed between postorbital–hypural body length and egg weight (cf. Fleming 1986) for either coho (r = 0.671, n = 6, P = 0.145) or chum salmon (r = −0.302, n = 6, P = 0.562).

Coho

Coho salmon eggs increased from 0 to 48% in weight, depending on the treatment, after 7 d of preservation (Table 1). Time before preservation had minimal effect on the weight of eggs, while egg state, preservative, and individual fish effects

TABLE 1. Means and standard deviations (in parentheses) of the ratios of waterhardened and/or preserved (7d) to initial fresh egg weights for coho and chum salmon.

Species	Time before preservation (h)	Fresh		Unfertilized–waterhardened			Fertilized–waterhardened		
		Stockard's	Formalin	None	Stockard's	Formalin	None	Stockard's	Formalin
Coho	3	1.055 (0.031) $n = 30$	0.999 (0.019) $n = 30$	1.257 (0.058) $n = 59$	1.488 (0.136) $n = 29$	1.167 (0.062) $n = 30$	1.274 (0.056) $n = 60$	1.481 (0.156) $n = 26$	1.180 (0.052) $n = 29$
	7	1.055 (0.031) $n = 28$	0.997 (0.016) $n = 30$	1.250 (0.053) $n = 60$	1.466 (0.140) $n = 28$	1.177 (0.055) $n = 29$	1.260 (0.054) $n = 60$	1.448 (0.121) $n = 29$	1.184 (0.053) $n = 29$
	24	1.057 (0.031) $n = 28$	1.004 (0.019) $n = 30$	1.248 (0.056) $n = 58$	1.499 (0.135) $n = 28$	1.184 (0.058) $n = 30$	1.243 (0.048) $n = 60$	1.466 (0.138) $n = 28$	1.188 (0.048) $n = 30$
	Times combined	1.055 (0.030) $n = 86$	1.000 (0.018) $n = 90$	1.252 (0.056) $n = 177$	1.484 (0.136) $n = 85$	1.176 (0.058) $n = 89$	1.259 (0.054) $n = 180$	1.464 (0.137) $n = 83$	1.184 (0.050) $n = 88$
Chum	3	1.048 (0.013) $n = 58$	1.003 (0.010) $n = 60$	1.170 (0.023) $n = 120$	1.358 (0.054) $n = 58$	1.120 (0.014) $n = 60$	—	—	—
Species combined		1.052 (0.024) $n = 144$	1.001 (0.015) $n = 150$	1.218 (0.060) $n = 297$	1.432 (0.125) $n = 143$	1.153 (0.052) $n = 149$	1.259 (0.054) $n = 180$	1.464 (0.137) $n = 83$	1.184 (0.050) $n = 88$

TABLE 2. Analysis of variance of the effects of preservation treatments on the size of coho salmon eggs. The analysis compares the ratio of preserved (7d) egg weight to initial fresh egg weight.

Effect	Degrees of freedom	F	Probability
State	2,10	114.35	<0.001
Preservative	1,5	134.34	<0.001
Fish[a]	5,216	385.42	<0.001
Time	2,10	3.94	0.055
State–preservative	2,10	110.36	<0.001
State–fish	10,216	52.89	<0.001
State–time	4,20	1.33	0.292
Preservative–fish	5,216	44.90	<0.001
Preservative–time	2,10	6.45	0.016
Fish–time	10,216	1.84	0.056
State–preservative–fish	10,216	6.44	<0.001
State–preservative–time	4,20	1.71	0.188
State–fish–time	20,216	1.83	0.019
Preservative–fish–time	10,216	0.67	0.755
State–preservative–fish–time	20,216	1.76	0.026

[a]Individual females.

TABLE 3. Analysis of variance of the effects of preservation on coho and chum eggs combined.

Effect	Degrees of freedom	F	Probability
Species	1,10	4.40	0.062
State	1,10	300.34	<0.001
Preservative	1,10	584.59	<0.001
Fish[a]	10,96	32.28	<0.001
Species–state	1,10	8.45	0.016
Species–preservative	1,10	8.32	0.016
State–preservative	1,10	126.63	<0.001
State–fish	10,96	16.14	<0.001
Preservative–fish	10,96	3.42	<0.001
Species–state–preservative	1,10	3.46	0.093
State–preservative–fish	10,96	5.68	<0.001

[a]Individual females.

were all highly significant (Table 2). We therefore reduced the model by eliminating the time before preservation.

Waterhardening caused a significant increase in egg weight ($t = 13.15$, df = 10, $P < 0.001$), and the extent of this effect was not altered by fertilization ($t = -0.12$, df = 10, $P = 0.907$; see Table 1). After preservation, waterhardened and fresh eggs still differed significantly (Stockard's: $t = 23.87$, df = 13, $P < 0.001$; formalin: $t = 11.26$, df = 8, $P < 0.001$).

Both preservatives generally caused an increase in egg weight, although the effect of Stockard's was more pronounced than that of formalin ($t = 11.59$, df = 5, $P < 0.001$). This difference was mainly a consequence of the fertilized–waterhardened ($t = 13.13$, df = 8, $P < 0.001$) and unfertilized–waterhardened egg states ($t = 14.29$, df = 8, $P < 0.001$). The effect of the preservative was reduced for fresh eggs ($t = 3.23$, df = 8, $P = 0.012$), where neither preservative produced a significant alteration in egg weight (Stockard's: $t = 2.15$, df = 10, $P = 0.057$; formalin: $t = 0.06$, $P = 0.957$). Not only did Stockard's solution result in a greater increase in egg weight than formalin, but it also resulted in significantly more burst eggs during preservation (chi-square = 7.86, df = 1, $P = 0.005$).

The individual fish effect and its related interactions were all highly significant across the experiment. The standard deviation of the ratio of preserved to initial fresh egg weight among individuals ranged from 0.012 for the fresh, formalin treatment

TABLE 4. Regression equations for the relationships between egg diameters, and egg diameter and egg weight, under different preservation treatments. Coho and chum salmon were combined, as were eggs preserved fresh in formalin and in Stockard's, because no significant differences existed between these regressions ($P > 0.05$). All regressions were highly significant ($P < 0.001$).

Dependent variable	Independent variable	Treatment	Regression equation	n	R^2
Shortest axis diameter	Longest axis diameter	Preserved fresh	$y = 0.68 + 0.894(x)$	60	0.932
		Waterhardened formalin	$y = 0.75 + 0.847(x)$	40	0.786
		Waterhardened Stockard's	$y = -0.01 + 0.897(x)$	40	0.674
Weight	Shortest axis diameter	Preserved fresh	$y = 0.64(x)^{2.937}$	60	0.981
		Waterhardened formalin	$y = 0.94(x)^{2.751}$	40	0.940
		Waterhardened Stockard's	$y = 3.46(x)^{2.101}$	40	0.857
Weight	Longest axis diameter	Preserved fresh	$y = 0.88(x)^{2.755}$	60	0.964
		Waterhardened formalin	$y = 0.82(x)^{2.744}$	40	0.909
		Waterhardened Stockard's	$y = 0.88(x)^{2.668}$	40	0.915
Weight	Average diameter	Preserved fresh	$y = 0.69(x)^{2.889}$	60	0.988
		Waterhardened formalin	$y = 0.65(x)^{2.901}$	40	0.976
		Waterhardened Stockard's	$y = 1.19(x)^{2.587}$	40	0.966
Weight	Volume[a]	Preserved fresh	$y = 5.35 + 1.034(x)$	60	0.988
		Waterhardened formalin	$y = 8.84 + 1.000(x)$	40	0.975
		Waterhardened Stockard's	$y = 33.02 + 0.881(x)$	40	0.924

[a] Conversion from diameter to volume following Alderdice et al. (1979).

to 0.144 for the fertilized–waterhardened, Stockard's treatment. Neither female body length nor fresh egg weight was significantly correlated with this ratio under any of the preservation treatments ($P > 0.05$). However, there were significant correlations between waterhardened egg weight and the ratio of preserved to initial fresh egg weight ($P < 0.01$). When individual fish were isolated and tested, the general results were unchanged.

Coho and Chum

The preservative effects on chum salmon eggs were similar to those on coho (Table 1). When the chum and coho data were compared directly the main species effect and the second-order species interaction were not significant, while the first-order species interactions were significant, although relatively weakly so (Table 3). Egg state, preservative, individual fish, and their interactions all continued to exhibit strong, statistically significant effects on egg weight (Table 3). Chum salmon exhibited a similar, but smaller individual fish effect than coho (range of SD of ratio of preserved to initial fresh egg weight = 0.004–0.045). No correlations existed between the ratio of preserved to initial fresh egg weight and female body length, fresh egg weight, and waterhardened egg weight. A direct comparison of the chum and coho data showed no significant difference in preservation response ($t = 2.096$, df = 10, $P = 0.063$).

There was no significant change in egg weight between the first measure of preserved weight at 7 d and that at 5 mo under any preservation treatment, except waterhardened Stockard's, which resulted in an average decrease of 1.2 mg (0.5%) ($t = 3.40$, df = 19, $P = 0.003$).

The repeatability of egg weight measurements for coho salmon was high across all treatments (range of $r = 0.938$–0.975), while that for chum salmon was lower (range of $r = 0.543$–0.789).

Measurement error was small for weight ($\bar{x} = 0.93$ mg, SD = 0.91) and diameters of longest ($\bar{x} = 0.05$ mm, SD = 0.05) and shortest axes ($\bar{x} = 0.05$ mm, SD = 0.05). However, egg diameter provided an apparently less accurate measure of egg size than weight (Table 4). Eggs varied in shape, causing regressions between egg diameter measured along the longest and shortest axes to be weak relative to the other relationships, although still highly significant ($P < 0.001$; Table 4). These relationships also varied among preservation treatments. The best linear correlate to egg weight of those investigated was the average of the shortest and longest axis diameters (Table 4). As with fresh egg weight, fresh egg diameter was not altered by preservation in 5% formalin (average diameter: $t = 0.046$, df = 9, $P = 0.964$). Since no significant differences ($P > 0.05$) existed between regression of coho and chum egg diameters, they were combined, as were fresh eggs preserved in formalin and those preserved in Stockard's. Other regressions differed significantly ($P < 0.01$) amongst one another, and thus, other preservation treatments resulted in significant increases in egg diameters (Table 4).

Discussion

We found that commonly used preservation techniques involving Stockard's solution or 5% buffered formalin increased the weight of individual fresh eggs by 0–82%, averaging 22%. Compared with 5% buffered formalin, Stockard's solution produced a greater alteration in egg weight and was less consistent in its effect. Stockard's also destroyed significantly more eggs, possibly as a consequence of the greater swelling associated with it. The largest alteration in egg weight occurred when eggs were waterhardened prior to preservation in Stockard's solution. Although Velsen (1980) recommended the use of Stockard's solution for external examination of egg development, it is clearly not ideal for studies of egg size variation.

The best preservation technique, which produced no significant alteration in egg weight, was to place fresh eggs directly into 5% buffered formalin. Although suitable for salmon (*Oncorhynchus*) eggs, Markle (1984) found that this method caused the collapse of pelagic fish eggs and that an unbuffered solution of 5% formalin was more suitable. Buffering prevents the lowering of pH often associated with fixation and maintains the solution near neutral pH (Snyder 1983). Although some investigators have used higher concentrations of formalin, for example 10%, a 5% solution appears adequate for fixation and preservation of salmon eggs. It effects a good compromise between decomposition by bacteria and autolysis at low concentration and demineralization at high concentration due to low pH (Tucker and Chester 1984). A low-concentration solution also minimizes noxious vapors when working with specimens.

Waterhardening caused a 23% increase in egg weight. Water crosses the egg membrane as a result of an osmotic gradient created by the perivitelline colloids released from the cortical alveoli (Potts and Rudy 1969; Laale 1980). This produces a pressurized, fluid-filled perivitelline space and robust egg membrane, which buffers the egg from distortion caused by the external environment (Groot and Alderdice 1985). Individual differences among coho salmon in reaction to preservation techniques may, in part, be a consequence of individual differences in water uptake during waterhardening.

Fertilization had no significant effect on waterhardened egg weight in coho salmon. Bilton (1970) obtained similar results with sockeye salmon (*O. nerka*). By contrast, preservation method does alter waterhardened egg weight. Preservation in Stockard's solution after waterhardening resulted in a further 20% increase in egg weight. Conversely, preservation in 5% formalin caused a 5% decrease in egg weight. However, Heming and Preston (1981) found no significant alteration in weight of fertilized–waterhardened chinook salmon (*O. tshawytscha*) eggs when preserved in 5% buffered formalin. This may be a species difference or a consequence of Heming and Preston anaesthetizing the eggs with tricaine methanesulfonate prior to preservation.

There was no species difference in the reaction of eggs to the preservation techniques, except that during waterhardening, coho eggs absorbed slightly more water relative to egg weight than chum eggs (Table 1). The egg membranes of chum salmon tend to be thicker than those of coho and contain an additional layer, the subinternus (Groot and Alderdice 1985), which may influence water uptake.

The repeatability measurement of egg weight among coho salmon indicates that variation among individuals is much greater than within individuals. This suggests that there will only be a slight rise in reliability using more than one egg measurement. Aulstad and Gjedrem (1973) came to similar conclusions concerning the repeatability of egg diameters in Atlantic salmon (*Salmo salar*). The lower level of egg weight repeatability in chum than in coho salmon may stem from low variability in egg weight among individuals used in this study.

Researchers have used various measures and methods of determining egg size without attempting to standardize their procedures. Egg diameter, the most commonly reported measure in the literature, is relatively inaccurate for nonspherical eggs. With swelling, salmon eggs change from an ellipsoidal to spherical shape, causing the relationship between short and long axes to vary (Table 4). Egg weight provides a more consistent and accurate measure of egg size. The use of egg weight also provides information for life history studies, where one is interested in the resource allocation to egg production and the trade-off between fecundity and egg size (Svardson 1949; Smith and Fretwell 1974; Winkler and Wallin 1987).

We therefore recommend that salmonid egg measurements be made by weight on fresh eggs or fresh eggs preserved in a 5% phosphate-buffered formalin solution. Studies reporting other common measurement and preservation techniques can be corrected using the data in Tables 1 and 4.

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Homing in Wild Sockeye Salmon (*Oncorhynchus nerka*) Populations as Inferred from Differences in Parasite Prevalance and Allozyme Allele Frequencies

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Although it is widely accepted that adult Pacific salmon (*Oncorhynchus* spp.) spawn in their natal stream, there are few quantitative estimates of homing precision in wild populations. The prevalence of two myxosporean parasites, *Myxobolus neurobius* and *Henneguya salminicola*, indicated very precise homing in certain sockeye salmon (*O. nerka*) populations in British Columbia (Long and Owikeno lakes on the central coast and Sproat, Great Central, and Henderson lakes on Barkley Sound, Vancouver Island). These populations were also sampled for electrophoretic differences at 23 loci. Genetic differences were found among all five populations, and estimates of straying from these data corroborated the parasitological evidence that straying is rare (<1%).

Il est universellement accepté que le saumon du Pacifique adulte (*Oncorhynchus* spp.) va frayer dans son cours d'eau natal, mais on dispose de peu de données quantitatives sur la précision de cet instinct de retour chez les populations sauvages. La prévalence de deux parasites à myxospore, *Myxobolus neurobius* et *Henneguya salminicola*, a indiqué la présence d'un instinct de retour très précis chez certaines populations de saumon nerka (*O. nerka*) en Colombie-Britannique (lacs Long et Owikeno sur la côte centrale et lacs Sproat, Great Central et Henderson dans la baie Barkley, sur l'île de Vancouver). On a également identifié des différences électrophorétiques à 23 locus chez ces populations. On a observé des différences génétiques chez les cinq populations et ces données nous ont permis d'estimer la proportion de poissons qui ne manifestent pas l'instinct de retour. Ces résultats indiquent que les «déviants» sont rares (<1%), ce que confirment les données sur les parasites.

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Pacific salmon (*Oncorhynchus* spp.) are known for their tendency to spawn in their natal stream (reviewed by Scheer 1939). However, it has long been known that some "stray" and spawn elsewhere. The consequences of straying have been discussed by various authors (Semko 1954; Hanamura 1966; Hasler 1966; Quinn 1984). Although the relative homing tendencies of different species have been compared in the literature (Hanamura 1966), accurate data needed to support such comparisons are not available and reliable estimates of straying in wild populations are scarce. Straying is an important consideration in fisheries management because it could influence stock assessment calculations, and also because it sheds light on the question "How serious is the loss of a particular stock?" (Larkin 1972). In addition, straying behavior provides some perspective on the importance of homing in salmon evolution (Quinn 1985).

Sockeye salmon (*O. nerka*) are suggested to have a particularly strong homing instinct or ability (Hanamura 1966; Semko 1954). A comprehensive study on homing by wild sockeye salmon to Cultus Lake, British Columbia, in the 1930s reported that no more than 0.6 and 1.5% of the adults entering the lake could have been strays in two years of study (Foerster 1936, 1968). Tagging studies on wild salmon are very laborious and expensive, and no major sockeye smolt tagging project for the purpose of studying homing has been undertaken since Foerster's work.

Naturally occurring "biological tags" have been used to identify some salmon populations (Awakura et al. 1982; Margolis 1982) and may yield valuable data on the precision of homing. For example, the prevalence of two myxosporean parasites, *Myxobolus neurobius* and *Henneguya salminicola*, is highly variable among sockeye populations, yet appears to be stable over time within a population. These parasites infect juvenile salmon in fresh water and remain in the fish throughout the period of marine residence. Thus, homing tendencies can be inferred in cases where the infection rates in adjacent river systems differ (Margolis 1965). Electrophoretic analysis of polymorphic enzymes has also been used to differentiate sockeye salmon populations (Grant et al. 1980; Utter et al. 1984; Wilmot and Burger 1985; Withler 1985). Significant differences in allele frequencies among neighboring populations are often interpreted as evidence of low rates of gene flow. For this reason, electrophoretic results can provide an independent check on inferences about homing drawn from patterns of parasite prevalence.

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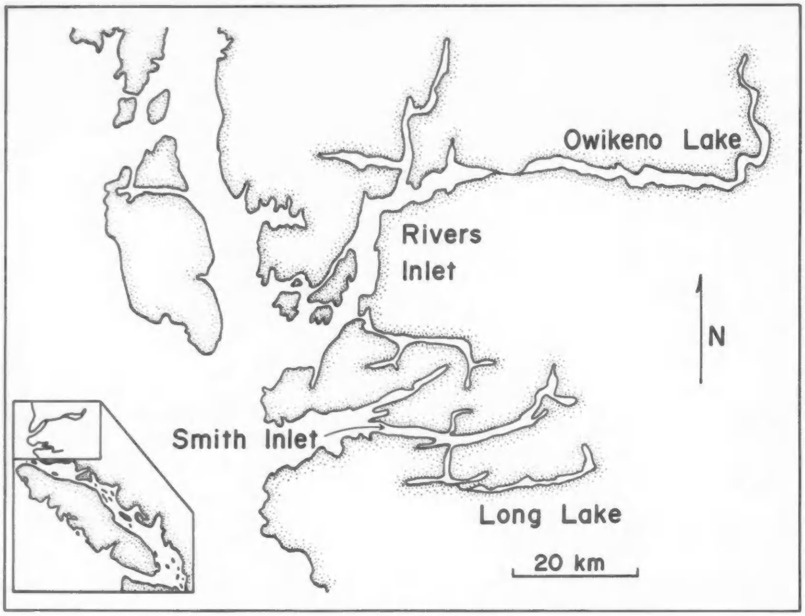

FIG. 1. Location of Owikeno and Long lakes.

This paper presents data on the parasite prevalence and electrophoretic characteristics of five wild sockeye salmon populations with different degrees of geographical isolation. The prevalence of *Myxobolus* and *Henneguya* in returning adult sockeye was used to estimate maximum straying rates among populations. These results were corroborated by examining differences in allele frequencies for eight polymorphic allozymes. In addition, a general model is presented which can be used to estimate maximum straying rates from parasite prevalence or other forms of differential rank data.

Methods

Population Groups

Electrophoretic and parasite prevalence data have recently been compiled for a large number of sockeye populations in British Columbia for the purpose of stock identification (L. Margolis, B. E. Riddell, and C. C. Wood, unpubl. data). From this data base, two groups of sockeye populations were selected in which variation in parasite prevalence among adjacent populations provided an opportunity to assess homing tendencies to lakes at two different levels of geographical isolation. The first group consists of populations that reside in Long and Owikeno lakes, situated on adjacent inlets on the central coast of British Columbia (Fig. 1). The spawning times of the adult sockeye in tributaries of the lakes overlap broadly. Smolts were collected at the outlets of these lakes for some years between 1977 and 1984 (Hyatt et al. 1984), and adult salmon were collected from the spawning grounds for some years between 1978 and 1984 (Table 1). The populations in the second group reside in three lakes tributary to Barkley Sound on the west coast of Vancouver Island. Sproat and Great Central lakes drain, via the Sproat and Stamp rivers, respectively, into the Somass River at the head of Alberni Inlet and are separated by a river distance of less than 25 km. Henderson Lake drains into Barkley Sound via the Henderson River and Uchuklesit Inlet approximately 40 km from the Somass River (Fig. 2). Spawning times in these three systems also overlap (Hyatt and Steer 1987). Both smolts and adults were collected at the outlets of Great Central Lake and Sproat Lake from 1977 to 1984. Smolts were collected from the outlet and adults from the spawning grounds in Henderson Lake from 1978 to 1984 (Table 1).

Parasitological Analysis

Myxobolus neurobius is detectable microscopically as spores in the hindbrain of both juvenile and adult salmon. A sensitive technique for detecting spores of *M. neurobius* involves digestion of the brain in a pepsin – hydrochloric acid solution, followed by centrifugation to concentrate the spores and microscopic examination of the sediment in the centrifuge tubes. The technique is a modification of that described by Anonymous (1984) for recovery of *Myxosoma cerebralis* spores from cranial cartilage. Although the technique is effective for concentrating and detecting fully formed spores, early stages of the parasite are probably digested. Hence, infections that have not reached the spore-forming stage will not be detected. It is also possible that infections with very few spores present might be overlooked.

Henneguya salminicola, another myxosporean, is not readily detectable in juvenile salmon but forms macroscopically visible cysts in the musculature of adults (Boyce et al. 1985). Boyce et al. (1985) demonstrated that *H. salminicola* is transmitted in fresh water and is acquired by juvenile salmon before their seaward migration. The slow development of the parasite accounts for the absence of visible cysts containing the identifiable spore stage in juvenile salmon.

TABLE 1. Prevalence of the brain parasite (*Myxobolus neurobius*) and muscle parasite (*Henneguya salminicola*) in smolting and adult sockeye. Adult samples usually comprised two or more brood years. Spawning escapement estimates are from unpublished Department of Fisheries and Oceans data.

			Myxobolus				*Henneguya*	
			Smolts		Adults		Adults	
Lake	Calendar year	Spawning escapement (thousands)	Number sampled	% infected	Number sampled	% infected	Number sampled	% infected
Long	1977	105	25	0.0	0	—	0	—
	1978	75	0	—	50	0.0	0	—
	1979	20	238	0.0	0	—	0	—
	1983	200	0	—	100	0.0	0	—
	1984	89	200	0.0	154	0.0	0	—
	1977–84	—	463	0.0	304	0.0	0	—
Owikeno	1978	383	300	88.7[a]	269	98.1	0	—
	1979	290	205	79.2[a]	380	96.8	0	—
	1983	636	0	—	150	100.0	0	—
	1984	214	0	—	100	99.0	0	—
	1978–84	—	505	84.0[b]	899	98.5[b]	0	—
Great Central	1977	201	25	0.0	15	0.0	0	—
	1978	113	100	0.0	245	0.8	0	—
	1979	234	697	0.4	900	2.7	174	0.6
	1980	142	460	0.9	1179	5.6	1165	0.6
	1981	213	506	0.0	1337	8.4	1336	0.5
	1982	169	250	0.4	679	7.8	0	—
	1983	357	574	1.2	1034	2.4	0	—
	1984	133	400	3.0	851	3.8	0	—
	1977–84	—	3012	0.7[b]	6240	3.9[b]	2675	0.6[b]
Sproat	1977	79	25	100.0	15	100.0	0	—
	1978	37	54	96.3	175	99.4	0	—
	1979	73	607	98.7	760	99.6	175	0.0
	1980	107	431	97.0	1330	99.9	1330	0.0
	1981	110	475	97.3	1181	99.9	1181	0.0
	1982	215	298	99.0	843	99.9	0	—
	1983	256	402	98.0	1127	99.7	0	—
	1984	90	500	99.4	781	99.9	0	—
	1977–84	—	2792	98.2[b]	6212	99.8[b]	2686	0.0
Henderson	1978	7	65	100.0	150	97.3	188	54.8
	1979	20	399	88.2	211	98.1	211	75.8
	1980	21	200	96.0	200	100.0	200	71.5
	1981	40	230	93.9	300	98.3	300	72.7
	1982	56	100	99.0	300	98.7	300	63.3
	1983	43	200	99.0	299	100.0	299	58.5
	1984	45	100	94.0	201	99.0	201	65.7
	1978–84	—	1294	95.7[b]	1661	98.8[b]	1699	66.0[b]

[a] These smolts had been preserved in formalin and the technique of examining the brains for *Myxobolus* was not as sensitive as the spore-concentrating technique described in the text for use with fresh or frozen specimens. Hence, infections with comparatively few spores could easily have been overlooked and the prevalence of *Myxobolus* underestimated within these samples.

[b] Unweighted mean.

Electrophoretic Analysis

Heart, liver, eye, and muscle tissues were collected from 100–200 live sockeye from each of the five study populations in 1983–84. All samples were frozen within 12 h of collection. Twenty-three loci were assayed using standard extraction and horizontal gel electrophoretic techniques (e.g. May et al. 1979), but only eight loci were polymorphic for these populations (Table 2). The system of nomenclature suggested by Allendorf and Utter (1979) has been used to designate loci and alleles.

Allele frequencies at each locus were determined from the ratio of the number of each allele observed to the total number of alleles examined. Differences in genotype frequencies between populations were tested by likelihood ratio (i.e. *G*-test, Sokal and Rohlf 1981); all polymorphic loci were considered simultaneously. Allele frequencies at the eight polymorphic loci were

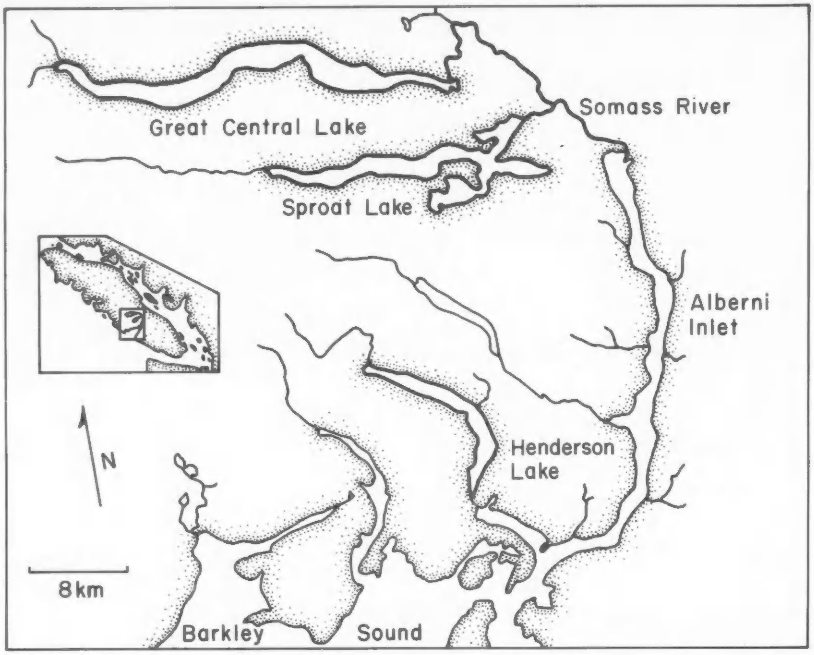

FIG. 2. Location of Great Central, Sproat, and Henderson lakes.

used to construct a similarity dendrogram (unweighted pair-group method of Sneath and Sokal (1973)) based on the unbiased genetic identity statistic (Nei 1978).

Estimation of Maximum Straying Rates

Straying rates were estimated by comparing parasite prevalence in samples of smolts and returning adults in neighboring lakes. We assumed that parasite prevalence among smolts is determined in the juvenile (freshwater) environment (Boyce et al. 1985; L. Margolis and T. E. McDonald, unpubl. data). Samples of adults returning to each lake were regarded as "mixtures," in terms of parasite prevalence, of smolt samples from neighboring populations from which adults may have strayed during their return migration. The mixing proportions of smolt types in the adult sample represent the net effect of (1) straying among populations, (2) infection of smolts subsequent to sampling the population, (3) failure to detect parasites in smolts at an early stage of infection, (4) differential mortality due to parasites, (5) loss of parasites, and (6) sampling error. Differential mortality and loss of parasites were assumed to be nonexistent or unimportant factors. We also assumed that the probability of a fish being infected by one parasite was independent of the probability of it being infected by the other parasite.

For Sproat and Great Central lakes, only data from age 1. adults (i.e. one freshwater check) were used to compare smolt and adult samples within brood years (Table 3). The possibility of straying from lakes besides Henderson Lake was discounted. Sockeye runs to Owikeno and Long lakes are by far the largest on the central coast of British Columbia (Starr et al. 1984), and the possibility of straying from other systems into these lakes was also discounted. Although 3 or 4 yr of data are available on brain parasite prevalance in both smolts and adults at each site (Table 1), a comparison between smolts and adults of the same brood year was not possible for the dominant freshwater age class. Consequently, for Owikeno and Long lakes, we compared parasite prevalence among smolts and adults for the pooled data in Table 1 only.

TABLE 2. Polymorphic enzymes used to infer genetic differences among sockeye populations.

Enzyme name	(EC number)	Locus	Tissue	Buffer[a]
Isocitrate dehydrogenase	(1.1.1.42)	Idh-3	Eye	AC
		Idh-4	Eye	AC
Lactate dehydrogenase	(1.1.1.27)	Ldh-4	Liver	RW
Malate dehydrogenase	(1.1.1.37)	Mdh-3	Muscle	AC
6-Phosphogluconate dehydrogenase	(1.1.1.44)	6-Pg	Muscle	AC
Phosphoglucoisomerase	(5.3.1.8)	Pgi-1	Muscle	RW
Phosphoglucomutase	(2.7.5.1)	Pgm-1	Heart	AC
		Pgm-2	Heart	AC

[a] AC = amine–citrate buffer (Clayton and Tretiak 1972); RW = discontinuous Tris, citric acid, lithium hydroxide, and boric acid buffer (Ridgway et al. 1970).

If the appropriate data are available, the mixing proportions for a three-stock smolt mixture can be determined using prevalence data for the two parasites by solving two simultaneous equations:

$$(1)\quad \begin{aligned} P_1 &= (1 - \Theta_{1,2} - \Theta_{1,3})p_1 + \Theta_{1,2}p_2 + \Theta_{1,2}p_3 \\ Q_1 &= (1 - \Theta_{1,2} - \Theta_{1,3})q_1 + \Theta_{1,2}q_2 + \Theta_{1,2}q_3 \end{aligned}$$

where P and Q refer to the two types of parasites, P_i and Q_i are the parasite prevalences among adults of population i, p_i and q_i

TABLE 3. Summary of parasite prevalence by brood year and estimated maximum straying rates for Great Central and Sproat lakes.

Lake	Brood year	Brood year escapement (thousands)	*Myxobolus* prevalence in age 1. returns: Number sampled	*Myxobolus* prevalence in age 1. returns: % infected	Estimated stock composition of age 1. returns (%) (±95% CI)[a]: Great Central	Estimated stock composition of age 1. returns (%) (±95% CI)[a]: Sproat	Estimated maximum straying rate (%) (±95% CI)[a]
Great Central	1976	138	936	6.0	94.0 (1.8)	6.0 (1.8)	13.8[b] (3.5)
	1977	101	1230	9.4	90.2 (2.1)	9.8 (2.1)	15.8 (2.8)
	1978	201	536	3.2	97.1 (1.6)	2.9 (1.6)	6.2 (3.3)
	1979	113	1035	2.4	98.3 (1.3)	1.7 (1.3)	13.8 (3.5)
Sproat	1976	47	1553	99.9	0.1 (0.2)	99.9 (0.2)	0.04[c] (0.06)
	1977	48	1020	100.0	−4.0 (6.1)	104.0 (6.1)	−1.8 (2.8)
	1978	79	893	99.9	−1.3 (0.9)	101.3 (0.9)	−0.5 (0.3)
	1979	37	918	100.0	−3.0 (1.9)	103.0 (1.9)	−0.9 (0.6)

[a] Assumes that change in parasite prevalence from smolts to adults was due entirely to straying and therefore represents the *maximum* straying rate. See text for method of calculation of these rates.

[b] Maximum rate of straying from Sproat to Great Central.

[c] Maximum rate of straying from Great Central to Sproat.

are the prevalences among smolts of population i, and Θ_{ij} is the proportion of fish originating in population j found in the adult sample from population i. Unfortunately, the muscle parasite could not be detected in smolts, and an explicit solution of these equations was not possible. We simplified the problem to permit solution by ignoring the possibility that straying occurs between Henderson Lake and Great Central or Sproat lakes. This seems justifiable because it is clear from the prevalence of the parasites in adult samples (Table 1) that straying from Henderson Lake to either Great Central or Sproat lakes and from Great Central Lake to Henderson Lake must be very low or nonexistent (see Results and Discussion). In view of these negligible straying rates, it seems probable that straying from Sproat Lake to Henderson Lake is also negligible.

The sampling distributions of the estimated mixing proportions, $\hat{\Theta}_{ij}$, for Great Central and Sproat lakes were determined from 100 Monte Carlo simulations using equation (2):

$$(2) \quad \Theta_{ij} = P_i - p_i/(p_j - p_i)$$

where P_i is defined as X_i/N_i and X_i follows the binomial distribution with parameters N_i and $\hat{P}_i$ to simulate sampling error; N_i and $\hat{P}_i$ refer to the number of fish sampled and the observed proportion of infected fish in the adult sample. The p_i were defined similarly using observed statistics from the smolt sample data.

Maximum straying rates were computed from the mixing proportion estimates by considering the relative magnitude of the donor and recipient populations. Annual escapement data were used for Great Central and Sproat lakes whereas average escapement data were used for Owikeno and Long lakes (Table 1). The maximum straying rate from population j to population i was defined as $\psi_{ij} = S_{ij}/\sum_j S_{ij}$ where S_{ij} represents the number of adults from population j returning to population i. Thus,

$$(3) \quad \psi_{ij} = \frac{\Theta_{ij}T_i}{\sum_i \Theta_{ij}T_i} = \frac{\text{strays from } j \text{ to } i}{\text{strays from } j \text{ to } i + \text{homers to } j}$$

since $S_{ij} = \Theta_{ij}T_i$ where T_i is the number of adults in all one-freshwater check (1.) age groups for the brood year in question returning to population i. No attempt was made to formally evaluate the significance of error in the escapement estimates, which were based on different sampling techniques at the five lakes. However, the populations of Sproat and Owikeno lakes may have been underestimated relative to the populations in Great Central and Long lakes, respectively. In both cases, the population estimates used resulted in higher straying estimates in Sproat and Owikeno lakes.

Results and Discussion

Parasitological Evidence for Homing

The difference in prevalence of *Myxobolus* in the brains of sockeye salmon from Long and Owikeno lakes (Table 1) indicated that essentially no Owikeno (infected) sockeye strayed into Long Lake. Because Owikeno Lake supports a much larger sockeye population than Long Lake, there is a good chance that Owikeno sockeye would have been detected in the Long Lake samples had they strayed. Since 2% of the samples in Owikeno Lake were uninfected, we cannot rule out the possibility that some straying from Long Lake occurred. However, there is no reason why the infection rate should be 100%, and other sockeye populations have *Myxobolus* prevalences between 0 and 100% (Bailey and Margolis 1987).

The *Myxobolus* results from the three Barkley Sound populations demonstrated that essentially no Great Central Lake sockeye (i.e. uninfected) entered either Henderson Lake (Table 1) or Sproat Lake (Table 3). Because the population of Great Central Lake is much larger than the other lakes (escapement estimates to Great Central Lake were approximately twice those to Sproat Lake in most of the sampling years and six times those to Henderson Lake), even a small proportion of strays from Great Central Lake would have been detected in the Henderson or Sproat samples by a reduced prevalence of *Myxobolus* infection in adults relative to smolts. The *Henneguya* results also indicated that Henderson Lake sockeye did not stray to Sproat Lake (no infected fish in samples totalling 2686 fish). Thus, Sproat Lake appeared to be free of strays from either of the two lakes tributary to Barkley Sound. The presence of 14 (0.5%) sockeye infected with *Henneguya* in Great Central Lake might suggest that a few Henderson Lake fish strayed to that lake. However, if those fish were strays from Henderson Lake, they would almost certainly ($p = 0.99$, Table 1) have been infected with *Myxobolus* in addition to *Henneguya*. Since all 14 fish were free of *Myxobolus*, it is virtually certain that they were native Great Central Lake stock. Moreover, it has been confirmed that *Henneguya* occurs within Great Central Lake; about 20% of coho salmon (*O. kisutch*) sampled from this lake in 1980 and 1981 were infected (Boyce et al. 1985).

Although neither Henderson Lake nor Great Central Lake sockeye appear to stray appreciably, the results for Sproat Lake were not as clear. The prevalence of *Myxobolus* cannot be used to assess straying between Henderson and Sproat lakes because almost all fish from both lakes were infected. *Henneguya* was absent in adult sockeye from Sproat Lake but occurred in only 66% of the Henderson Lake fish. Without data on the prevalence of *Henneguya* in smolts from Henderson Lake, it is not possible to determine from *Henneguya* prevalence data whether the nonparasitized Henderson Lake adults were strays from Sproat Lake. The extent to which Sproat Lake sockeye stray into Great Central Lake is confounded by two types of errors, both of which would tend to inflate the putative straying levels presented in Table 3. First, some smolts were infected with *Myxobolus* when they left Great Central Lake, but others may also have been infected without having the detectable spore stage at that time, or they may have had so few spores as to have been overlooked. Based on recent experiments, this proportion can be as high as 3% of the smolts leaving Great Central Lake. When Great Central Lake smolts captured at the outlet of the lake in 1984 were held in salt water for 4–5 mo (the estimated maximum time required for spore formation), an additional 1–3% of smolts were infected with *Myxobolus* spores compared with controls examined at the time the smolts were captured (L. Margolis and T. E. McDonald, unpubl. data). The second factor confounding estimates of straying by Sproat Lake fish into Great Central Lake is a consequence of the geography of the watershed. Smolts emigrating from Great Central Lake must swim down the Somass River, which receives water from Sproat Lake below its confluence with the Sproat River. Cage experiments have demonstrated that as many as 5–6% of the Great Central Lake smolts can acquire the parasite after being held for 1 wk in the Somass River at its confluence with the Sproat River (L. Margolis and T. E. McDonald, unpubl. data). Therefore, it is possible that some of the infected sockeye returning to Great Central Lake became infected after they left the lake as smolts. This does not mean that no Sproat Lake sockeye enter Great Central Lake, merely that our ability to use *Myxobolus* to ascertain their numbers is limited by the rates of detection error and possible infection in the Somass River.

Electrophoretic Evidence for Homing

Likelihood ratios of genotype frequencies (Table 4) at the eight polymorphic loci were highly significant ($p < 0.001$) for all pairwise comparisons of the five populations (Table 5). The similarity dendrogram (Fig. 3) illustrates their relative divergence based on the unbiased genetic identity statistic (Nei 1978). The similarity of Owikeno and Great Central lakes is probably coincidental, and is at least partly due to the choice of distance measure. Dendrograms derived from two other distance measures, the likelihood ratio (Table 5) and the chord distance (Cavalli-Sforza and Edwards 1967), suggest that Owikeno and Long lakes form one cluster, Great Central and Sproat lakes form another, and Henderson Lake remains distinct.

These genetic differences imply that gene flow among the populations must be small. The level of gene flow can be estimated if these alleles are assumed to be neutral to selection, and if mutation rates are known. We used two indirect procedures (described by Slatkin 1985) for estimating m, the proportion of a breeding population replaced by migrants from another population. First, we estimated coancestry coefficients, $\hat{\theta}_w$, for each pair of populations using all eight polymorphic loci and the weighting method recommended by Reynolds et al. (1983). From each coancestry coefficient, we estimated the corresponding value of Nm (where N is the effective population size for the pair of populations in aggregate) using the expression

$$(4)\quad (Nm)_{\text{est}} = \frac{1}{4}\left[\frac{1}{\hat{\theta}_w} - 1\right]$$

which is derived from an infinite island model of gene flow (e.g. Slatkin 1985). This approach ignores the effect of mutation. Estimated values for θ_w and Nm are summarized in Table 6. We calculated upper limits on the value of m by deliberately choosing low values for N. One half of the lowest escapement recorded in Table 1 was taken as the effective population size for each population; these values were summed to obtain a value of N for each pair of populations. Using this procedure, all estimates of m were less than 0.0002. In our second approach, we calculated m from Nei's genetic distance measure, D (Table 5), using the relationship

$$(5)\quad \exp(-D) = m/(m + \mu)$$

suggested by Nei (1975) where μ, the mutation rate, was assumed to be 2×10^{-6}. All estimates of m using eq. 5 were less than 0.0018. It should be noted that both approaches rely on untested assumptions and relatively few loci have been examined. Thus, these estimates should be interpreted cautiously. Nevertheless, the level of gene flow inferred from the available electrophoretic data is much lower than the maximum levels estimated from the parasitological results.

Two populations could sustain high straying rates and remain genetically distinct only if the strays were less likely to reproduce than the native stock (e.g. if there was discrimination against strays on the spawning grounds), or if the offspring of

TABLE 4. Allele frequencies at polymorphic loci for samples obtained from returning adults 1983–84. Numbers in parentheses designate alleles (Allendorf and Utter 1979).

		Population				
Locus	(Allele)	Owikeno	Long	Great Central	Sproat	Henderson
Pgm-1	Sample size	200	203	111	132	129
	(100)	0.273	0.530	0.239	0.383	0.004
	(null)	0.727	0.470	0.761	0.617	0.996
Pgm-2	Sample size	199	184	136	182	149
	(100)	0.857	0.829	0.879	0.799	0.977
	(120)	0.143	0.171	0.121	0.201	0.023
Idh-3	Sample size	200	184	143	183	154
	(100)	1.000	1.000	1.000	0.984	1.000
	(130)	0.000	0.000	0.000	0.016	0.000
Idh-4	Sample size	196	184	143	183	154
	(100)	0.992	1.000	0.979	1.000	1.000
	(162)	0.008	0.000	0.021	0.000	0.000
Ldh-4	Sample size	200	184	143	183	154
	(100)	0.970	0.986	1.000	0.997	1.000
	(115)	0.030	0.014	0.000	0.003	0.000
Mdh-3	Sample size	100	100	143	180	154
	(100)	1.000	0.990	0.976	0.992	0.968
	(120)	0.000	0.010	0.024	0.008	0.032
6-Pg	Sample size	100	100	136	183	104
	(100)	1.000	1.000	0.915	0.850	1.000
	(94)	0.000	0.000	0.085	0.150	0.000
Pgi-1	Sample size	100	82	76	128	111
	(100)	1.000	1.000	1.000	1.000	0.986
	(65)	0.000	0.000	0.000	0.000	0.014

TABLE 5. Matrix of unbiased genetic distance (Nei 1978) between stocks based on data in Table 4. Numbers in parentheses are chi-square values (with 11 df) for likelihood ratio. Double asterisks indicate that populations are significantly different ($p < 0.005$).

Population						
Number	Name	1	2	3	4	5
1	Owikeno	0.0000 (0)	—	—	—	—
2	Long	0.0090 (68.3**)	0.0000 (0)	—	—	—
3	Great Central	0.0011 (49.3**)	0.0130 (97.9**)	0.0000 (0)	—	—
4	Sproat	0.0048 (80.6**)	0.0059 (72.2**)	0.0039 (42.1**)	0.0000 (0)	
5	Henderson	0.0089 (137.8**)	0.0351 (255.7**)	0.0067 (106.6**)	0.0219 (221.2**)	0.0000 (0)

the strays were less likely to survive than the offspring of native stock. Regarding the first possibility, there is currently no evidence that mate choice by sockeye salmon is influenced by stock of origin, although the ability to discriminate between populations exists (Groot et al. 1986). Regarding the second possibility, there is some evidence that non-native stock may not survive as well as locally adapted stock (Ricker 1972; Withler 1982; Brannon and Hershberger 1984). Even so, the genetic differences corroborate our parasitological results, and it seems safe to conclude that little if any straying occurs among these populations.

Comparison with Other Studies of Salmon Homing

The parasite prevalence data (Tables 1 and 3) indicate that, in general, over 99% of sockeye that survive to adulthood and escape the fishing fleet return to their nursery lake in Barkley Sound or in the Smith/Rivers Inlet area. This figure exceeds the

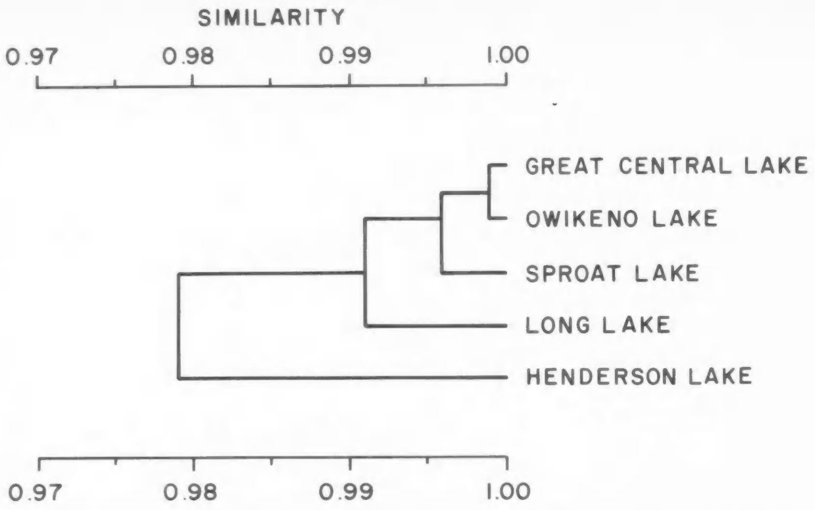

FIG. 3. Similarity dendrogram for the five sockeye populations examined based on allele frequencies at eight polymorphic loci using Nei's unbiased genetic identity (Nei 1978) and the unweighted pair-group method (Sneath and Sokal 1973).

TABLE 6. Matrix of coancestry coefficients between stocks (Reynolds et al. 1983) based on data in Table 4. Numbers in parentheses are estimates of *Nm* (i.e. the product of effective population size and migration rate) for each pair of populations.

Population						
Number	Name	1	2	3	4	5
1	Owikeno	0.0000 (—)	—	—	—	—
2	Long	0.078 (2.96)	0.0000 (—)	—	—	—
3	Great Central	0.010 (24.75)	0.096 (2.34)	0.0000 (—)	—	—
4	Sproat	0.037 (6.51)	0.040 (6.00)	0.028 (8.68)	0.0000 (—)	—
5	Henderson	0.149 (1.43)	0.346 (0.473)	0.129 (1.69)	0.225 (0.861)	0.0000 (—)

estimate of 95% homing used by Hasler and Scholz (1983) for Pacific salmon in general. It should be noted, however, that homing to the nursery lake is not the same as homing to the natal stream. For example, Long Lake has two productive spawning streams and Owikeno Lake has at least a dozen. The results of the present study do not pertain to homing within the lake itself. The only evidence for homing to the natal stream by sockeye is from experimental displacements of adults from spawning grounds. Such studies generally reveal that about 95% of the displaced fish repeat their original choice (Hartman and Raleigh 1964; McCart 1970; Varnavskiy and Varnavskaya 1985). However, McCart (1970) presented evidence that repeat-choice homing varied greatly among streams within Babine Lake, B.C. In general, the use of displacement studies for quantitative estimates of natal stream homing is questionable (Ricker 1972).

There are relatively few quantitative studies on other species of salmon that can be compared with the present results on sockeye salmon. Coho salmon returning to two small, unstable California coastal streams had high (27 and 15%) straying rates (Shapovalov and Taft 1954). In contrast with this pattern, coho returning to three hatcheries on Vancouver Island, B.C., showed virtually no evidence of straying to non-natal hatcheries (Quinn and Tolson 1986). Spring chinook salmon (*O. tshawytscha*) originating in the Cowlitz River Hatchery on the Columbia River rarely (1.4%) strayed to other rivers (Quinn and Fresh 1984). Different patterns of homing among populations and species may have both genetic and environmental bases. High variation in incubation and rearing environments between nearby streams may exact a higher mortality rate on the offspring of strays than would occur in more similar streams (Quinn 1984). However, certain rivers may also be easier to distinguish from each other based on their chemical characteristics. Salmon are generally believed to imprint on the odors of their natal stream in spring, yet typically return to spawn in summer or fall. Thus, the odors guiding home stream choice must persist over seasons and years. Large lakes might have more stable chemical characteristics than ephemeral tributaries, but little is known about the nature of the chemicals used by homing salmon (Hasler and Scholz 1983). Additional quantitative estimates of homing, particularly in wild populations in different types of river systems, will be needed to evaluate the relative importance of genetic and environmental factors influencing homing.

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Feeding Habits and Daily Ration of Walleye Pollock (*Theragra chalcogramma*) in the Eastern Bering Sea, with Special Reference to Cannibalism

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Dwyer, D. A., K. M. Bailey, and P. A. Livingston. 1987. Feeding habits and daily ration of walleye pollock (*Theragra chalcogramma*) in the eastern Bering Sea, with special reference to cannibalism. Can. J. Fish. Aquat. Sci. 44: 1972–1984.

Stomach contents of 3098 walleye pollock (*Theragra chalcogramma*) collected from the eastern Bering Sea during 1981–83 were examined to (1) describe the diet as influenced by area, season, and fish size, (2) estimate daily ration, and (3) examine the magnitude of cannibalism. Small pollock fed mainly on copepods and shifted to euphausiids and fish with increased size. Stomach contents (percent body weight) were highest in summer and autumn. Laboratory-derived estimates of gastric evacuation rates and age-structured estimates of abundance were used in conjunction with the field data to estimate daily ration of pollock in the eastern Bering Sea. Mean annual daily ration was calculated to be about 0.3% body weight per day. Daily ration was lowest in spring and lower in the northwestern region of the eastern Bering Sea than in the southeastern region. Cannibalism occurred in all seasons, but consumption was highest on age 0 pollock in the southeastern area during autumn. It is estimated that about 400 billion age 0 pollock were consumed annually by larger pollock in the eastern Bering Sea.

On a examiné le contenu stomacal de 3098 goberges de l'Alaska (*Theragra chalcogramma*) prises dans la partie orientale de la mer de Béring entre 1981 et 1983 en vue de: 1) décrire le régime selon l'endroit, la saison et la taille du poisson, 2) calculer la ration quotidienne et 3) étudier l'ampleur du cannibalisme. Les petites goberges se nourrissent principalement de copépodes, puis se tournent vers les Euphausiacés et les poissons à mesure qu'elles grandissent. Le contenu stomacal (% du poids corporel) est plus élevé en été et en automne. Le taux d'évacuation gastrique et l'estimation de l'abondance en fonction de l'âge calculés au laboratoire ainsi que les données recueillies dans le milieu naturel ont été utilisés pour calculer la ration quotidienne des goberges dans la partie orientale de la mer de Béring. Selon les calculs, la ration quotidienne annuelle moyenne est d'environ 0,3 % du poids corporel par jour. La ration quotidienne est à son plus bas au printemps et plus faible dans la région du nord-ouest que dans la région du sud-est de la partie orientale de la mer de Béring. Le cannibalisme s'observe à toutes les saisons mais il se produit surtout aux dépens des goberges d'âge 0 dans la région du sud-est pendant l'automne. On estime qu'environ 400 milliards de goberges d'âge 0 sont consommées annuellement par les plus grosses goberges dans la partie orientale de la mer de Béring.

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Multispecies approaches to the problems of fisheries management require a more complete understanding of trophic interactions in marine systems. The walleye pollock (*Theragra chalcogramma*), with an estimated exploitable biomass in the eastern Bering Sea of about 10 million metric tons (t) (Bakkala et al. 1987), is a major predator on zooplankton and fish and an important food resource for fish, marine mammals, and birds (Smith 1981). As a keystone species, a thorough understanding of the pollock's trophic role is essential. Sensitivity analyses have shown the importance of food composition and feeding rates of pollock to Bering Sea ecosystem models (Livingston 1985; Knechtel and Bledsoe 1983).

In several previous studies (Takahashi and Yamaguchi 1972; Mito 1974; Bailey and Dunn 1979), euphausiids, copepods, and juvenile pollock were identified as major prey items for pollock. However, none of these studies is comprehensive. The present study was designed to provide detailed information on seasonal, areal, and size-related variability in feeding. In addition, measured rates of gastric evacuation are used in conjunction with stomach contents data to estimate daily ration.

Of special interest is the apparently widespread occurrence of cannibalism among pollock in the eastern Bering Sea. Smith (1981) suggested that density-dependent mortality due to cannibalism on juvenile pollock may dampen variability in recruitment. Walline (1983) concluded that predation on juvenile pollock rather than availability of food to larvae appears to control year-class strength. Similar results have been obtained from models designed to simulate the population dynamics of pollock (Laevastu and Favorite 1981; Knechtel and Bledsoe

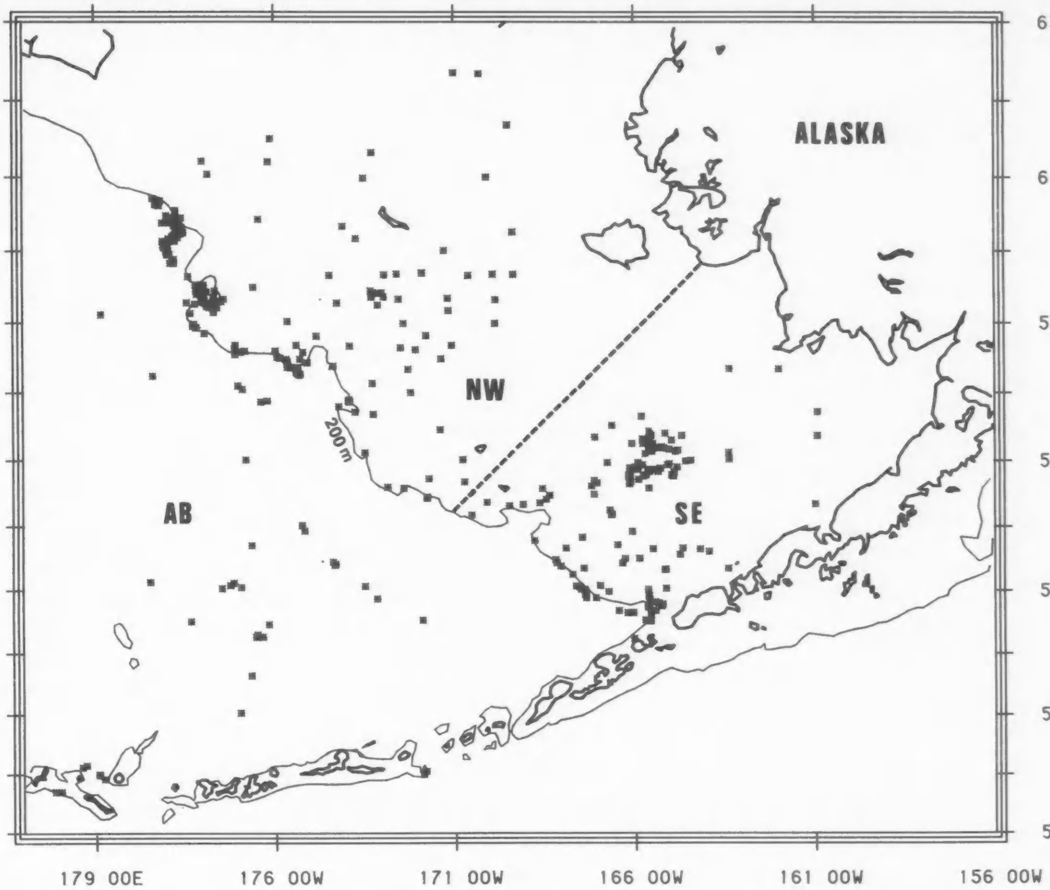

FIG. 1. Sampling locations for walleye pollock collected from the eastern Bering Sea during July 1981 – June 1983.

1983). In this study we calculate the magnitude of cannibalism based on estimates of daily ration and age-structured biomass of the pollock.

Methods and Materials

Stomachs were collected from 3098 walleye pollock (16–78 cm fork length (FL)) over a period of 2 yr (July 1981 – June 1983) from the eastern Bering Sea (Fig. 1; Table 1). Sampling gear included both bottom and midwater trawls from research and commercial fishing vessels, and samples were collected throughout the 24-h day. Stomachs were removed at sea and placed in muslin bags labeled with the location of capture and length, sex, and sexual maturity of the fish. Individual fish weights were calculated using a length–weight relationship developed for pollock in the eastern Bering Sea (Pereyra et al. 1976). Fish showing evidence of regurgitation (i.e. food in the mouth or throat, or a flaccid stomach) were not included in the sample. Stomachs were preserved in 10% formalin and returned to the laboratory for analysis. In the laboratory the samples were transferred to 50% isopropyl alcohol. Contents were identified to the lowest taxonomic level possible and enumerated. Wet weights were recorded after the contents were blotted with paper towels. Whole fish in the stomachs were measured to the nearest millimetre standard length (SL).

Stomach samples were divided into four groups according to season of capture: spring (15 March – 14 June), summer (15 June – 14 September), autumn (15 September – 14 December), and winter (15 December – 14 March). These seasonal data sets were further divided among three geographic areas based on evidence of stock structure (Lynde et al. 1986; Hinckley 1987): the continental shelf and slope to the northwest of the Pribilof Islands (NW), the shelf and slope to the southeast of these islands (SE), and the eastern Aleutian Basin (AB) (from 179°W eastward to the slope). These samples were further separated into four predator length groups (<30, 30–39, 40–49, and ≥50 cm).

Gastric evacuation rates of pollock were determined in the laboratory. Pollock (37–45 cm) were collected by hook and line from Puget Sound (Washington) and held in covered net-pens at the Manchester Aquaculture Laboratory, Manchester, WA. Average water temperatures were 8.5–9.0°C. Fish were held for 3 d without food to ensure that stomachs were empty and were then anesthetized with tricaine methanesulfonate (MS-

TABLE 1. Sampling distribution of 3098 stomachs (including empty stomachs) of walleye pollock collected from the eastern Bering Sea. NW refers to the shelf/slope area to the northwest of the Pribilof Islands; SE refers to the shelf/slope area to the southeast of the Pribilof Islands; AB refers to the Aleutian Basin.

		Fish length (cm)				
Season	Area	<30	30–39	40–49	≥50	Total
Spring	NW	12	143	101	83	339
	SE	10	149	160	78	397
	AB	0	7	12	19	38
	Total	22	299	273	180	774
Summer	NW	127	173	109	131	540
	SE	6	45	53	6	110
	AB	0	2	36	7	45
	Total	133	220	198	144	695
Autumn	NW	12	112	105	103	332
	SE	45	126	139	112	422
	AB	0	1	2	3	6
	Total	57	239	246	218	760
Winter	NW	45	216	148	114	523
	SE	1	4	16	121	142
	AB	0	50	84	70	204
	Total	46	270	248	305	869
Total	NW	196	644	463	431	1734
	SE	62	324	368	317	1071
	AB	0	60	134	99	293

222) and force-fed a measured meal of either two whole juvenile pollock (8–10 g) or an equivalent weight of whole euphausiids (*Euphausia pacifica*). All food items had been previously frozen and were thawed prior to use. Fish were sacrificed at intervals after feeding. Contents from the stomachs were recovered, blotted, and weighed to the nearest 0.01 g.

The depletion of stomach contents (W) with time (t) was described with an exponential relationship (Elliott 1972; Cochran and Adelman 1982):

$$(1)\quad W_t = W_0 e^{-Rt}$$

where R is the instantaneous rate of gastric evacuation. An exponential model was used to describe the relationship between R and temperature (T) after Elliott (1972):

$$(2)\quad R = ae^{bT}.$$

Durbin et al. (1983) found that the slope (b) of the linearized equation was constant over prey type and had an average value of 0.115, but the intercept (a) varied by prey type. The model may therefore be written as

$$(3)\quad W_t = W_0 e^{-(ae^{0.115T})t}.$$

Our laboratory experiments on evacuation rates of juvenile pollock and euphausiids as prey were preformed at seawater temperatures of 9.0 and 8.5°C, respectively. The temperatures were entered as constants in equation (3), which was the logarithmic weighted least-squares regression model for estimating a at these fixed temperatures (Draper and Smith 1981; Wonnacott and Wonnacott 1984). Equation (2) was used to calculate gastric evacuation rate R at other temperatures (assuming $b = 0.115$). Average bottom temperatures for the two areas during each of the four seasons were estimated from Ingraham (1983).

The instantaneous rate of gastric evacuation R was used in the Elliott and Persson (1978) model to estimate daily ration:

$$(4)\quad \Sigma C_t = \frac{Rt}{1 - e^{-Rt}} \sum_{i=1}^{m} S_i (1 - e^{-Rt}) = 24\bar{S}R.$$

In this model, t is the duration of each interval (i) over which stomach samples are collected, S is the mean stomach content as percent of fish body weight, m is the total number of intervals in a 24-h day, and ΣC_t is daily ration in terms of percent body weight (% BWD). $\bar{S}$ ($= \Sigma S_i / m$) is the mean amount of food in the stomach over a 24-h period.

An estimate of the total amount of young pollock consumed by the entire population of pollock was calculated according to Mehl and Westgard (1983):

$$(5)\quad C_{ijk} = \mathrm{DR}_{ijk} \times D_i \times B_{ijk} \times P_{ijk}$$

where C_{ijk} is the consumption (by weight) of young pollock by pollock belonging to size group j during season i in area k, DR_{ijk} is the daily ration (% BWD) represented by fish prey, D_i is the number of days in season i, B_{ijk} is the biomass of pollock, and P_{ijk} is the proportion (by weight) of fish prey which were juvenile pollock. Areas used for calculation of daily ration were NW and SE. Pollock predators were divided into size groups of <40 and ≥40 cm for these calculations.

Biomass (B) of pollock in the eastern Bering Sea was estimated from data collected during resource assessment surveys conducted by the Northwest and Alaska Fisheries Center (NWAFC) during 1982 (Bakkala et al. 1985). Since a large portion of the pollock population is found off the bottom (Traynor 1986), results of a hydroacoustic midwater trawl survey were combined with results from the bottom trawl survey. Total biomass was apportioned among the two size groups (<40 and ≥40 cm) based on length–frequency measurements taken by both bottom and midwater trawl surveys.

To compare the age composition of prey pollock (P) with survey estimates, the prey fish were separated into age groups based on Smith (1981): age 0 = 1–130 mm; age 1 = 131–220 mm; age 2 = 221–300 mm; age 3 = 301–350 mm.

The average length of prey pollock in each age group within season/area/size subsets was determined from measurable fish found in the stomachs. Average weights of prey pollock were calculated for 0- to 1-yr-old fish as

$$(6)\quad W = (5.9 \times 10^{-6})L^{3.1}$$

and for 1- to 3-yr-old pollock as

$$(7)\quad W = 0.0075L^{2.977}$$

where W is weight (grams) and L is length (centimetres) from equations of Walline (1983) and Pereyra et al. (1976), respectively.

Results

Diet

Effects of season, area, and predator size

Prey composition in the stomach contents varied with season, area, and predator size (Fig. 2). Euphausiids were an especially important component of the diet of all but the smallest predator size-classes in the SE and NW areas in spring and summer. Copepods were prevalent in the diet of small fishes in the NW

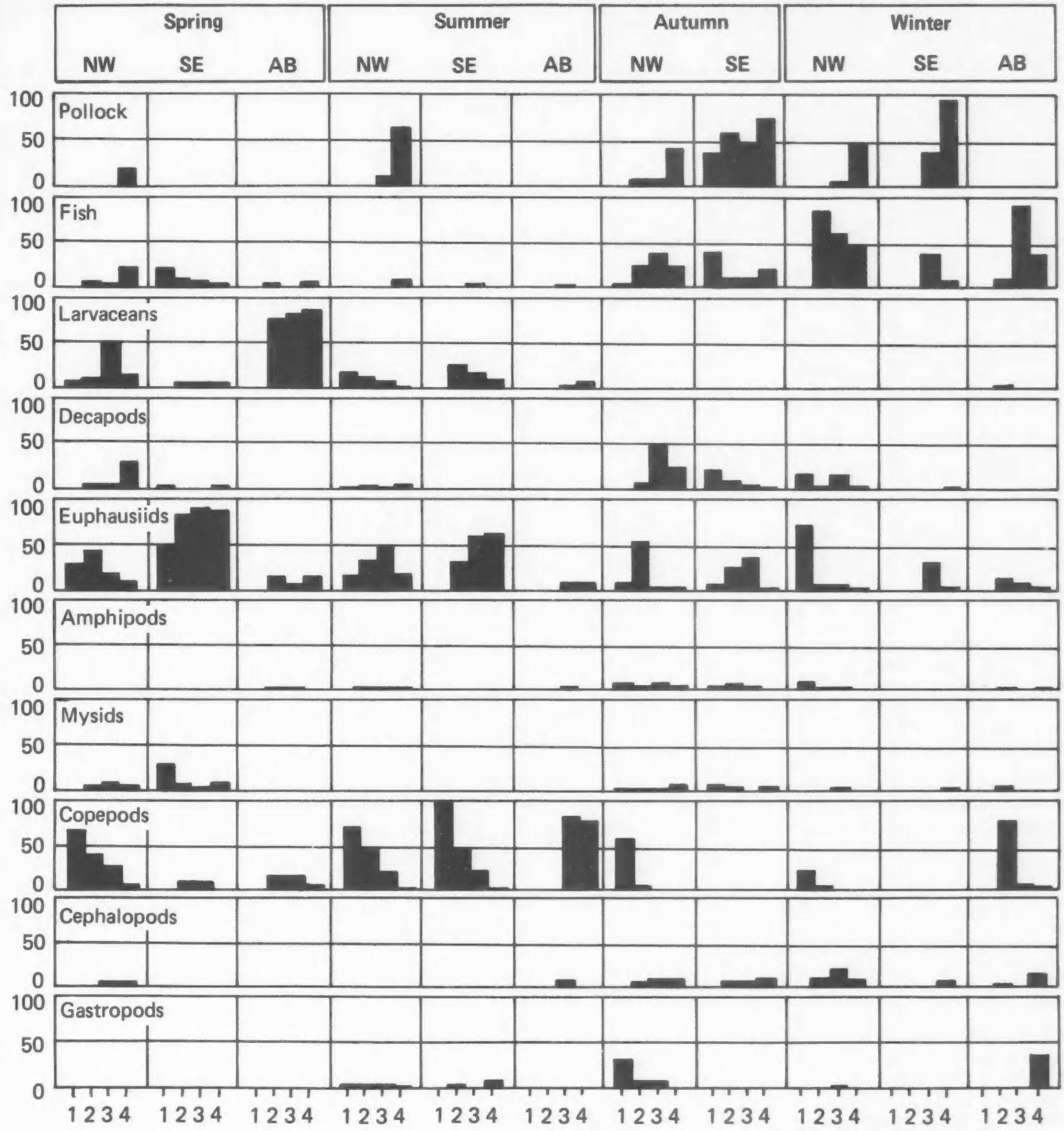

FIG. 2. Percent by weight of major prey categories in the diet of walleye pollock by season, area, and fish size. Subsets with fewer than five stomachs are not included. Size categories: 1 = <30 cm; 2 = 30–39 cm; 3 = 40–49 cm; 4 = ≥50 cm.

area in spring and in the NW and SE areas in summer. In the AB area, larvaceans dominated the diet in spring, copepods in summer, and fishes (mainly myctophids) in winter. Size-related shifts in the diet from invertebrates to fishes were noted and were especially evident in some seasons. In spring and summer, consumption of copepods decreased with increasing fish size, while consumption of euphausiids and fishes, especially by the predators >50 cm in length in the NW area, increased. Similar size-related shifts to piscivory were noted in autumn and winter. The occurrence of pollock as prey will be discussed separately below.

The percentage of empty stomachs tended to be highest in winter for all sizes of fish (Fig. 3) in areas pooled. Stomachs for the NW area were more often empty than those collected from the SE area. The trend in mean average weight of stomach contents (% BW) was inversely related to that of empty stomachs (Fig. 4). The lowest stomach weights occurred in winter, with the highest values generally occurring in summer.

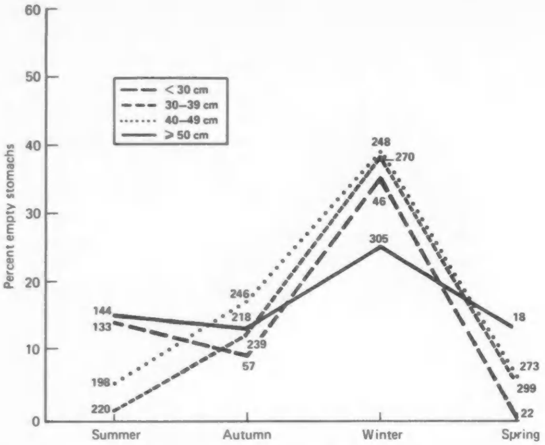

FIG. 3. Percent of walleye pollock stomachs which were empty by season and fish size. Numbers are sample sizes.

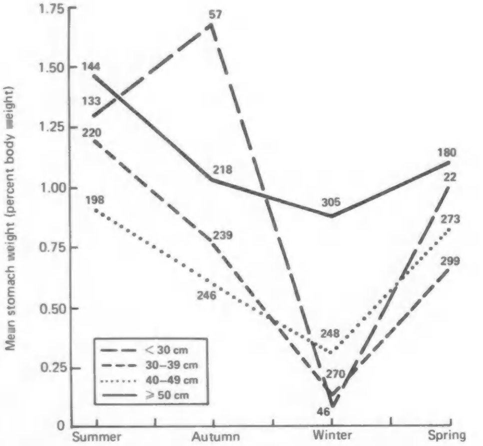

FIG. 4. Mean weight of stomach contents, as percent body weight, by season and fish size. Numbers are sample sizes.

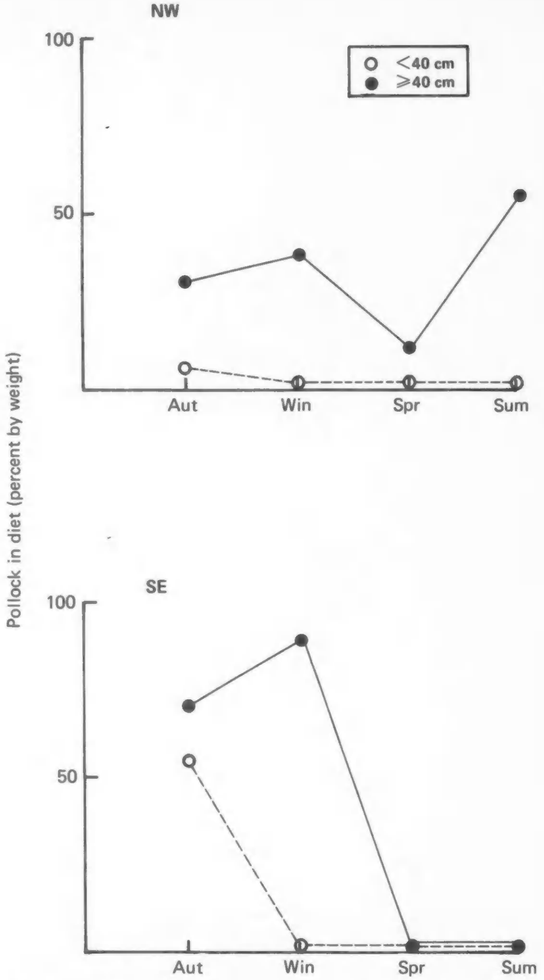

FIG. 5. Percent by weight of prey walleye pollock in the diet of walleye pollock by area, season, and fish size.

Cannibalism

Seasonal, area, and size-related differences in cannibalism (percent by weight of diet composed of young pollock) were found (Fig. 5). Cannibalism was not observed in the AB region. In the SE area, young pollock was a major component of diets of both predator size groups (<40 and ≥40 cm) in autumn (55–71%) and was the major item in the diet of larger predators in winter (88%). There was minimal cannibalism in spring and summer in the SE area. In the NW areas there was little cannibalism by the smaller predators except in autumn. Cannibalism by the larger pollock was prevalent in autumn, winter, and summer.

The size range of pollock consumed differed markedly between the NW and the SE. In the SE, most young pollock found in stomachs in autumn fell in the 60–90 mm size range, corresponding to age 0 fish (Fig. 6). In this same area in winter, most pollock consumed were also age 0 fish, 70–100 mm in length. By contrast, young pollock consumed by predators collected in the NW represented several age groups. In autumn, prey pollock ranged in size from 40 to 200 mm, indicating that both age 0 and age 1 fish were consumed. Although few pollock were found in the stomachs of pollock collected from the NW in winter, most were age 1 fish (160–230 mm). The widest size range, 20–330 mm, occurred among pollock consumed during summer in the NW. Although age groups 0–3 appear to be represented here, the majority of these prey pollock were age 1 fish (130–240 mm).

Estimates of Consumption

Daily ration

Gastric evacuation rates were obtained from the laboratory experiments for both juvenile pollock and euphausiids as prey (Fig. 7). Estimates of *a* obtained from weighted least-squares regression of equation (3) were 0.0143 for juvenile pollock as prey at 9°C and 0.0078 for euphausiids as prey at 8.5°C. Estimates of gastric evacuation rates, *R*, at eastern Bering Sea

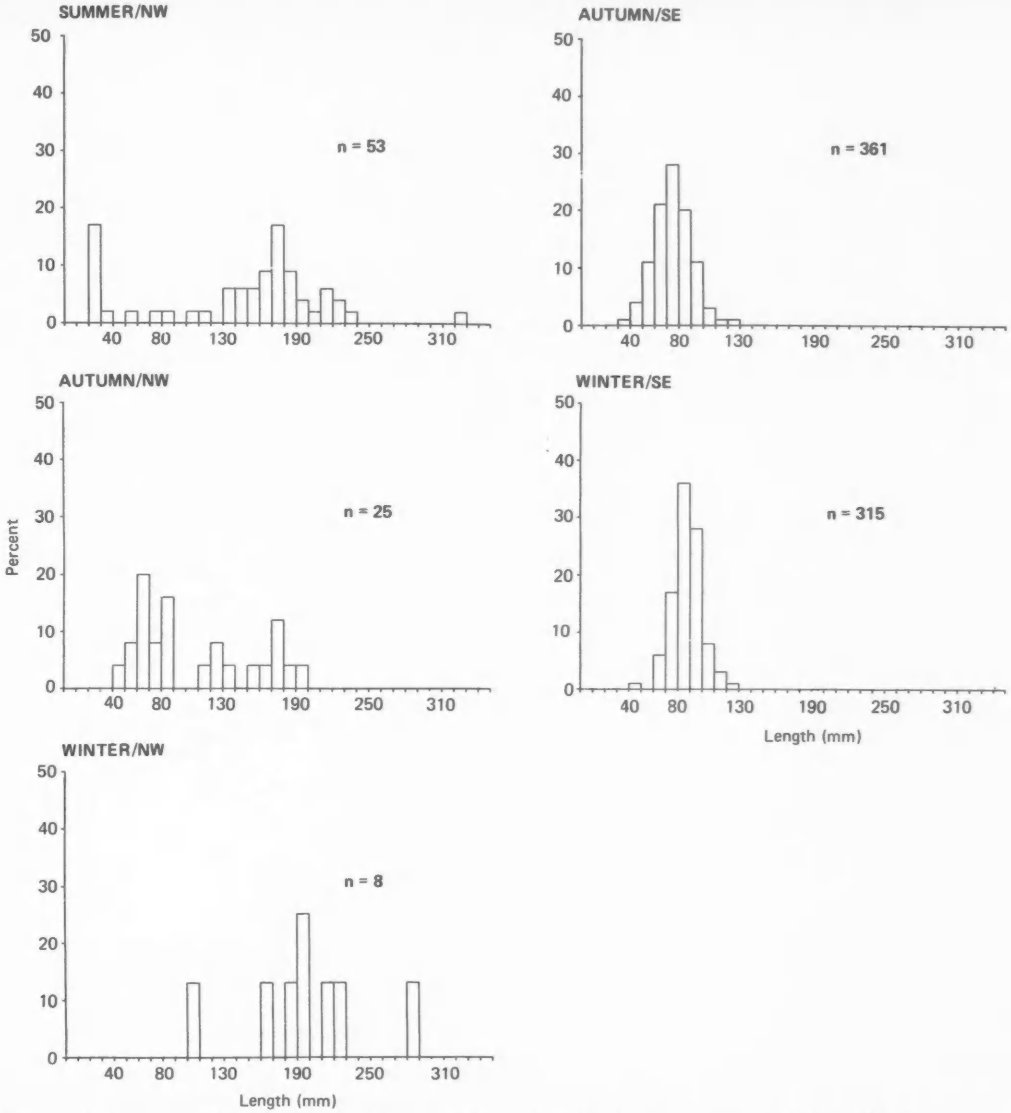

FIG. 6. Length–frequency distribution of measurable prey walleye pollock found in the stomachs of walleye pollock by season and area.

bottom temperatures of around 2–3°C (using equation (2) and assuming $b = 0.115$) averaged 0.02 for juvenile pollock as prey in both NW and SE on an annual basis and 0.01 for euphausiids as prey (see Appendix).

Estimates of daily ration obtained by combining information on rates of gastric evacuation and mean stomach content weights ranged from a high of 0.62% BWD (fish <40 cm) in the SE during autumn to a low of 0.05% BWD (fish ≥40 cm) in the NW during winter (Table 2; see Appendix). There were insufficient data to calculate daily ration in the AB region. Annual means of daily ration calculated with all geographical data pooled were 0.26% for predators <40 cm and 0.32% for predators ≥40 cm.

Population estimates of cannibalism

The amount of young pollock consumed over the entire year by pollock of all sizes in the eastern Bering Sea was estimated to be approximately 2.7 million t (Table 3). Sixty-five percent of this amount was consumed in the SE.

Of prey pollock consumed, 97% (402.1×10^9) were age 0 fish (Table 4). Of the age 0 fish, 95% (382.4×10^9) were consumed in the SE and 64% (242.9×10^9) were consumed during autumn. Pollock <40 cm accounted for about one half (121.2×10^9) of the total consumption in the SE during this season. Large numbers (139.5×10^9) of age 0 pollock were also consumed by pollock ≥40 cm during winter in the SE. Due to the small number of pollock <40 cm collected from the SE in

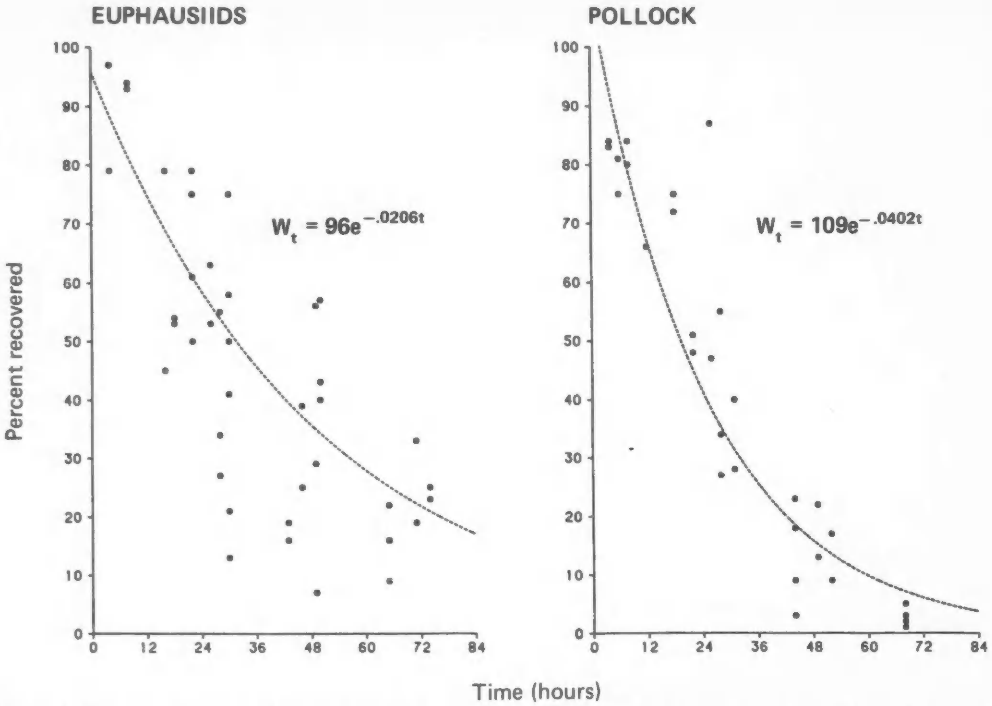

FIG. 7. Results of laboratory gastric evacuation experiments, using euphausiids and juvenile walleye pollock as prey items. The curve is a fitted exponential decay function of the form $W_t = W_o e^{-bt}$; $r^2 = 0.84$ and 0.94 for euphausiid and pollock curves, respectively.

TABLE 2. Estimated daily ration (% body weight per day) of pollock in the eastern Bering Sea by season, area, and predator size.

		Season			
Area	Size (cm)	Summer	Autumn	Winter	Spring
NW	<40	—[a]	0.62	—[a]	0.23
	≥40	—[a]	0.45	0.59	0.19
NW	<40	0.31	0.10	0.05	0.06
	≥40	0.44	0.19	0.25	0.09

[a] Insufficient data at one or more time periods to make calculations.

winter, it was not possible to estimate the impact of their wintertime cannibalism. As noted above, we were not able to make daily ration estimates from the SE area in summer, but cannibalism appeared to be minimal in this season and area (Fig. 2).

About 58% (19.7×10^9) of the pollock consumed by pollock in the NW were age 0 fish. Most of those remaining were age 1 fish (12.7×10^9), while much smaller numbers of age 2 (1.4×10^9) and age 3 (0.2×10^9) fish were eaten. By contrast, 100% (382.4×10^9) of the pollock consumed by pollock in the SE were age 0 fish.

Discussion

Pollock can consume a variety of prey organisms, but depend on a relatively limited number for most of their food. There were marked seasonal effects on the prey composition, e.g. feeding on copepods and euphausiids in spring and summer and on fishes in autumn and winter. These trends are apparently related to seasonal changes in prey abundance (Cooney 1981). In general, stomach content weights were highest in summer and lowest in winter. Size-related shifts in the diet from small invertebrates to fishes were also noted and were especially prevalent from summer–winter. Similar shifts toward piscivory with increasing size have been noted in other gadoid fishes, including Atlantic cod (*Gadus morhua*) (Daan 1973), Pacific whiting (*Merluccius productus*) (Livingston 1983), and silver hake (*Merluccius bilinearis*) (Langton 1982).

Prey consumption of fish can be calculated either by the stomach content method we employed or from an energetic approach. For comparison, the bioenergetic equation of Jones (1978) was used to calculate the daily ration of pollock:

$$F = 0.008W^{0.8} \exp(0.081T) + 1.27GW^{0.15}$$

where F is the rate of food consumption (grams per day), W is the body weight (grams) (427 g for an average age 4 pollock (Niggol 1982)), G is the growth rate (grams per day) (0.43 $g \cdot d^{-1}$ for an age 4 pollock, estimated from Niggol (1982)), and T is the temperature (3°C for the eastern Bering Sea). The resulting food requirements of pollock would be 2.65 $g \cdot d^{-1}$ for a 427-g pollock, or 0.6% BWD. Alternatively, Smith et al. (1986) calculated the relationship between food intake and growth for juvenile pollock at 3°C:

$$G = -0.264 + 1.03F$$

where G is growth (% BWD) and F is consumption (% BWD).

TABLE 3. Estimates of walleye pollock consumption due to cannibalism in the eastern Bering Sea.

Area (k)	Season (i)	Size group (fish length cm) (j)	DR_{ijk}	D_i	B_{ijk}(t)	P_{ijk}	C_{ijk}(t)
NW	Spring	≥40	0.0003	92	2 365 680	0.46	30 035
	Summer	≥40	0.0026	92	2 365 680	0.92	520 601
	Autumn	<40	0.0004	91	3 562 320	0.23	29 824
		≥40	0.0012	91	2 365 680	0.57	147 249
	Winter	≥40	0.0022	90	2 365 680	0.44	206 098
						Total	933 807
SE	Autumn	<40	0.0048	91	981 436	0.84	360 101
		≥40	0.0038	91	1 822 563	0.83	523 101
	Winter	≥40	0.0057	90	1 822 563	0.94	878 876
						Total	1 762 078
						Grand total	2 695 885

NOTE: Calculations are based on equation (5):

$$C_{ijk} = DR_{ijk} \times D_i \times B_{ijk} \times P_{ijk}$$

where C = amount of pollock consumed (t), DR = daily ration of pollock (fish prey only), B = biomass of pollock (potential predators), and P = proportion of fish prey which was pollock.

TABLE 4. Estimated numbers ($\times 10^9$) of walleye pollock consumed by walleye pollock in the eastern Bering Sea, with a breakdown of prey pollock by age group. C_{ijk} is the amount of pollock consumed (t) during season i by fish size group j in area k; P_{0-3} is the proportion (by weight) of prey pollock belonging to age groups 0–3, respectively; W_{0-3} is the mean weight (g) of prey pollock belonging to age groups 0–3, respectively.

				Age group 0			Age group 1			Age group 2			Age group 3		
Area	Season	Size group (cm)	C_{ijk} (t)	P_0	W_0	Number eaten	P_1	W_1	Number eaten	P_2	W_2	Number eaten	P_3	W_3	Number eaten
NW	Spring	≥40	30 035	—	—	—	1.00	64.17	0.468	—	—	—	—	—	—
	Summer	≥40	520 601	0.01	0.96	5.423	0.72	47.56	7.881	0.14	99.47	0.733	0.13	282.24	0.240
	Autumn	<40	29 824	1.00	3.33	8.956	—	—	—	—	—	—	—	—	—
		≥40	147 249	0.17	5.05	4.957	0.83	44.90	2.722	—	—	—	—	—	—
	Winter	≥40	206 098	0.02	11.07	0.372	0.53	65.80	1.660	0.45	140.08	0.662	—	—	—
					Total	19.708			12.731			1.395			0.240
SE	Autumn	<40	360 101	1.00	2.97	121.246	—	—	—	—	—	—	—	—	—
		≥40	523 101	1.00	4.30	121.651	—	—	—	—	—	—	—	—	—
	Winter	≥40	878 876	1.00	6.30	139.504	—	—	—	—	—	—	—	—	—
					Total	382.401			0			0			0
					Grand total	402.109		Total	12.731		Total	1.395		Total	0.240

Using the same growth rate as in the previous example ((0.43 g/427 g) 100 = 0.1% BWD), the estimate of food consumption is 0.35% BWD. These estimates of daily ration (0.3–0.6% BWD) are somewhat higher than those obtained by stomach content analyses (0.25–0.32% BWD).

Both methods of determining daily ration involve sources of error, and it is not clear which might be more reliable. Elliott and Persson (1978) asserted that direct estimates of food consumption generally require fewer assumptions than estimates from theoretical energy requirements or growth rates. Bioenergetic models may suffer from errors in parameter estimation (Bartell et al. 1986), but they are useful to explore the effects of parameter variations (Olson and Boggs 1986). Besides results of the above comparisons, we have reason to believe that our estimates from stomach contents are conservative. The techniques of starvation (i.e. withholding food for 3 d before the gastric evacuation experiments) and force-feeding may have resulted in conservative estimates of daily ration. Both of these techniques may cause depression of gastric evacuation rates in some fishes (Swenson and Smith 1973; Jones 1978). However, other studies indicate that short periods of starvation have little effect on rate of gastric evacuation (Brett and Higgs 1970; Elliott 1972). Our own (unpublished) results show a minimal (2–5%) effect of force-feeding. Loss of prey weight in stomachs due to fixation in 10% formalin and 50% alcohol is unknown, but reasonably could range from 10 to 30%. A further bias towards conservatism would occur if regurgitated stomachs were not recognized as such, and were categorized as empty stomachs.

The mean daily ration obtained in our study is low compared with daily ration estimates of other gadoid fishes: 0.6–1.7% BWD for Atlantic cod (Daan 1973; Jones 1978; Durbin et al. 1983), 0.6–4.6% BWD for silver hake (Durbin et al. 1983), 1.0–2.8% BWD for haddock (*Melanogrammus aeglefinus*) (Jones 1978), and 2.5% BWD for Pacific whiting (Livingston 1983). However, the studies mentioned above were performed at water temperatures of 6–12°C, while daily rations reported for pollock in this study are based on water temperatures of 2–3°C. Since temperature is related to metabolic requirements and to the evacuation rate of stomach contents, our lower daily ration estimates are expected.

Daily ration estimates of pollock from the NW area were lower than those from the SE area. In general, the NW area is characterized by colder subsurface temperatures and greater seaward extent of ice in winter. The lower daily ration estimates in the NW compared with the SE resulted from lower mean stomach content weights and colder temperatures used in our model of gastric evacuation. Lower daily ration in the NW area is corroborated by recent results showing that growth rates (Lynde et al. 1986) and reproductive output (Hinckley 1987) in the NW are lower than those in the SE area.

The results of this study, in addition to those of earlier studies (Takahashi and Yamaguchi 1972; Mito 1974; Bailey and Dunn 1979), indicate that cannibalism is a major feature of the feeding habits of pollock in the eastern Bering Sea. Our study showed that consumption of juveniles was highest in the SE area of the Bering Sea. The majority of cannibalism occurred in autumn and winter, accounting for 62 and 34% of the total numbers of fish consumed, respectively. Ninety-six percent of the young pollock consumed were age 0 fish.

Heavy cannibalism appears to be a unique feature for pollock over the eastern Bering Sea shelf/slope that is not observed elsewhere in the NE Pacific, including the AB area (Okada 1986; this study), the Gulf of Alaska (Smith et al. 1978), or in bays in southeastern Alaska (Clausen 1983). In the eastern Bering Sea, horizontal and vertical overlap of adults and juveniles, as a necessary condition for cannibalism, must be extensive. The consumption of age 0 fish in the SE generally agrees with what is known about the location of spawning and of later distribution of young-of-the-year fish (Smith 1981; Traynor 1986). A large number of age 1 pollock were cannibalized in the NW area, which is consistent with what is known about their distribution in years of available data (1975, 1979–82); 77–98% of age 1 pollock were found in the NW (Bakkala and Alton 1986).

Cannibalism is minimal in spring and early summer because in these months, age 0 pollock are in the mixed layer, generally the upper 40 m (Nishiyama et al. 1986), and adults are below 60 m (Traynor 1986). In autumn, age 0 fish recruit to deeper water (Traynor 1986). In these seasons, they coincide with adults and are vulnerable to cannibalism (Francis and Bailey 1983).

The total estimated number of age 0 pollock consumed was 402.4×10^9 fish. Based on the results of a hydroacoustic midwater trawl survey conducted by the NWAFC in 1982, Traynor (1986) estimated a range of 106×10^9 to 201×10^9 age 0 pollock in the eastern Bering Sea. However, he noted that this estimate could be an order of magnitude low due to high escapement from trawls (J. J. Traynor, NWAFC, Seattle, WA, pers. comm.). The total abundance of age 0 fish was estimated by similar methods in 1984 at around 680×10^9 (J. J. Traynor, pers. comm.). Since juvenile pollock have also been shown to be an important prey item for other abundant fish species in the eastern Bering Sea (Livingston et al. 1986), as well as for marine mammals (Kajimura and Fowler 1984) and birds (Smith 1981), it seems likely that either the abundance estimates for age 0 pollock are low or that the consumption estimates are high. Given the conservative daily ration values from this study, we believe that the consumption estimates derived from them are low. Besides parameters used in estimating daily ration, our calculations of the magnitude of cannibalism are undoubtedly sensitive to estimates of stock biomass. In general, 95% confidence intervals on biomass estimates from bottom trawl surveys are ±25% and from acoustic surveys are ±12% due to sampling error alone (Bakkala et al. 1987).

The results of our study are in general agreement with those of ecosystem simulations. Current results of PROBUB (described in Laevastu and Larkins 1981), a biomass-based simulation model which uses estimates of growth and removal rates of pollock and other components of the eastern Bering Sea food web, put annual consumption of pollock by pollock at 26.6% BW, 0.07% BWD. This value compares well with the cannibalism estimate of 0.09% BWD obtained in the present study.

Given the problem of estimating abundance of age 0 fish, it is difficult to determine the effect of cannibalism on year-class strength. However, we can indirectly evaluate this problem. Mito (1974) found that pollock made up 70–80% (by weight) of the diet of pollock sampled in the eastern Bering Sea during autumn of 1972; at the same time, the biomass of adult pollock appeared to be relatively high (Bakkala et al. 1987). Considering these data, cannibalism might have significantly affected the 1972 year-class; however, it became a very strong one (Yamaguchi 1986). The proportion by weight of age 0 pollock in the stomach contents of pollock ≥40 cm during autumn was larger in 1982 than in 1981 (57 and 24% of stomach contents, respectively); adult biomasses in both years were similarly high but the 1982 year-class was four times more abundant than the 1981 year-class as 1-yr-olds (Bakkala et al. 1987). Consequently, it appears that the relative degree of cannibalism by adult pollock may be an indicator of the abundance of age 0 pollock.

Compensatory mortality due to cannibalism is one factor that can give rise to dome-shaped stock–recruitment curves (Ricker 1954). In some freshwater lakes, predation on juvenile fishes is the major determinant of recruitment levels, although this predation mortality can be either depensatory (Forney 1971, 1977) or compensatory (Tarby 1974). For walleye pollock in the eastern Bering Sea, the appearance of strong year-classes in spite of heavy cannibalism suggests depensatory predation mortality. This could happen if unusually high survival of larvae results in juvenile abundances that overwhelm the predatory capacity of adults (Peterman and Gatto 1978). Heavy cannibalism on already low numbers of juveniles after poor larval survival could greatly impact recruitment. These preliminary observations are speculative and conflict with the hypotheses that cannibalism dampens recruitment variability (Smith 1981) and that predation on juveniles controls year-class strength (Walline 1983).

Continued monitoring of adult abundances, year-class strengths, and the extent of cannibalism could yield valuable information on population regulation mechanisms of pollock. We suggest further research on the effect of environmental factors on the overlap of juveniles and adults, energy requirements of pollock, and sampling techniques to estimate the abundance of age 0 fish.

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Appendix

Mean amount of food (% BW) in the stomachs of walleye pollock sampled, with estimates of gastric evacuation rate, R, and daily ration, DR (% BW per day). $\bar{S}$ is the mean amount of food in the stomach over a 24-h period; n is the number of stomachs examined (including empty stomachs); BW = body weight; DR = 24 $\bar{S}R$.

					Stomach contents (% BW)		
Area	Season	Size group (cm)	Time	n	Total food	Fish prey	Other prey
NW	Spring	<40	24–03	24	0.261	0.000	0.261
			03–06	13	0.344	0.008	0.336
	Temp. = 1.85°C		06–09	40	0.223	0.015	0.208
			09–12	29	0.153	0.016	0.138
			12–15	21	0.128	0.028	0.099
	R (fish) = 0.0177		15–18	11	0.568	0.000	0.568
			18–21	13	0.215	0.004	0.212
	R (other) = 0.0096		21–24	4	0.097	0.000	0.097
					$\bar{S} = 0.249$	$\bar{S} = 0.009$	$\bar{S} = 0.240$
					DR = 0.00	DR = 0.00	DR = 0.06
NW	Spring	≥40	24–03	14	0.090	0.001	0.089
			03–06	23	0.412	0.077	0.335
	Temp. = 1.85°C		06–09	16	0.463	0.297	0.166
			09–12	36	0.620	0.013	0.607
			12–15	15	0.423	0.028	0.395
	R (fish) = 0.0177		15–18	19	0.018	0.000	0.018
			18–21	31	0.396	0.029	0.367
	R (other) = 0.0096		21–24	30	0.259	0.092	0.167
					$\bar{S} = 0.335$	$\bar{S} = 0.067$	$\bar{S} = 0.268$
					DR = 0.09	DR = 0.03	DR = 0.06
NW	Summer	<40	24–03	28	0.744	0.006	0.738
			03–06	29	1.596	0.000	1.596
	Temp. = 2.23°C		06–09	35	0.865	0.005	0.861
			09–12	60	1.300	0.000	1.300
			12–15	46	0.975	0.000	0.975
	R (fish) = 0.0185		15–18	48	1.093	0.001	1.092
			18–21	39	1.507	0.002	1.505
	R (other) = 0.0101		21–24	15	2.113	0.001	2.112
					$\bar{S} = 1.274$	$\bar{S} = 0.002$	$\bar{S} = 1.272$
					DR = 0.31	DR = 0.00	DR = 0.31
NW	Summer	≥40	24–03	34	0.677	0.102	0.576
			03–06	13	1.627	1.498	0.129
	Temp. = 2.23°C		06–09	35	1.784	1.216	0.568
			09–12	51	1.387	1.086	0.302
			12–15	47	1.240	0.196	1.045
	R (fish) = 0.0185		15–18	34	1.646	0.274	1.372
			18–21	21	0.859	0.368	0.491
	R (other) = 0.0101		21–24	5	1.593	0.000	1.593
					$\bar{S} = 1.352$	$\bar{S} = 0.593$	$\bar{S} = 0.759$
					DR = 0.44	DR = 0.26	DR = 0.18
NW	Autumn	<40	24–03	6	0.341	0.290	0.051
			03–06	15	0.152	0.001	0.151
	Temp. = 2.25°C		06–09	19	0.330	0.036	0.294
			09–12	9	0.007	0.000	0.007
			12–15	15	0.400	0.229	0.171
	R (fish) = 0.0185		15–18	22	0.606	0.140	0.466
			18–21	16	0.175	0.007	0.168
	R (other) = 0.0101		21–24	22	0.634	0.066	0.568
					$\bar{S} = 0.331$	$\bar{S} = 0.096$	$\bar{S} = 0.235$
					DR = 0.10	DR = 0.04	DR = 0.06

APPENDIX. (*Continued*)

					Stomach contents (% BW)		
Area	Season	Size group (cm)	Time	*n*	Total food	Fish prey	Other prey
NW	Autumn	≥40	24–04	5	0.265	0.196	0.069
			04–08	29	1.006	0.255	0.751
	Temp. = 2.25°C		08–12	28	0.406	0.265	0.141
			12–16	61	0.574	0.220	0.354
			16–20	24	0.295	0.258	0.037
	R (fish) = 0.0185		20–24	61	0.646	0.375	0.271
					$\bar{S} = 0.532$	$\bar{S} = 0.262$	$\bar{S} = 0.270$
					DR = 0.19	DR = 0.12	DR = 0.07
NW	Winter	<40	24–03	25	0.061	0.008	0.053
			03–06	46	0.082	0.064	0.018
	Temp. = 2.15°C		06–09	16	0.074	0.029	0.045
			09–12	35	0.121	0.049	0.073
			12–15	51	0.229	0.212	0.017
	R (fish) = 0.0183		15–18	34	0.057	0.023	0.033
			18–21	10	0.169	0.111	0.057
	R (other) = 0.0100		21–24	44	0.217	0.180	0.037
					$\bar{S} = 0.126$	$\bar{S} = 0.084$	$\bar{S} = 0.042$
					DR = 0.05	DR = 0.04	DR = 0.01
NW	Winter	≥40	24–03	28	0.326	0.269	0.057
			03–06	39	1.393	1.294	0.099
	Temp. = 2.15°C		06–09	28	0.386	0.088	0.298
			09–12	37	0.331	0.172	0.159
			12–15	33	0.724	0.695	0.029
	R (fish) = 0.0183		15–18	39	0.588	0.492	0.096
			18–21	26	0.640	0.497	0.143
	R (other) = 0.0100		21–24	32	0.723	0.569	0.154
					$\bar{S} = 0.639$	$\bar{S} = 0.510$	$\bar{S} = 0.129$
					DR = 0.25	DR = 0.22	DR = 0.03
SE	Spring	<40	24–03	20	0.426	0.005	0.421
			03–06	17	0.620	0.000	0.620
	Temp. = 2.40°C		06–09	2	0.105	0.000	0.105
			09–12	22	0.614	0.009	0.604
			12–15	13	1.981	0.180	1.800
	R (fish) = 0.0188		15–18	25	0.674	0.254	0.420
			18–21	30	0.974	0.000	0.974
	R (other) = 0.0103		21–24	30	1.528	0.302	1.226
					$\bar{S} = 0.865$	$\bar{S} = 0.094$	$\bar{S} = 0.771$
					DR = 0.23	DR = 0.04	DR = 0.19
SE	Spring	≥40	24–03	12	0.489	0.009	0.481
			03–06	10	0.022	0.000	0.022
	Temp. = 2.40°C		06–09	4	0.110	0.000	0.110
			09–12	25	0.349	0.000	0.349
			12–15	47	1.650	0.000	1.650
	R (fish) = 0.0188		15–18	52	1.504	0.115	0.389
			18–21	36	1.212	0.000	1.212
	R (other) = 0.0103		21–24	52	1.764	0.037	1.727
					$\bar{S} = 0.763$	$\bar{S} = 0.020$	$\bar{S} = 0.743$
					DR = 0.19	DR = 0.01	DR = 0.18
SE[a]	Summer	<40					
SE[a]	Summer	≥40					
SE	Autumn	<40	24–03	16	0.650	0.217	0.434
			03–06	2	2.693	2.172	0.521
	Temp. = 3.59°C		06–09	46	0.873	0.553	0.321
			09–12	17	2.023	0.554	1.469
			12–15	16	0.948	0.843	0.105

APPENDIX. (*Concluded*)

					Stomach contents (% BW)		
Area	Season	Size group (cm)	Time	n	Total food	Fish prey	Other prey
	R (fish) = 0.0216		15–18	16	1.461	1.411	0.049
			18–21	37	1.194	0.782	0.412
	R (other) = 0.0118		21–24	21	1.496	0.948	0.548
					$\bar{S} = 1.417$	$\bar{S} = 0.935$	$\bar{S} = 0.482$
					DR = 0.62	DR = 0.48	DR = 0.14
SE	Autumn	≥40	24–04	26	0.244	0.105	0.139
			04–08	31	1.514	1.456	0.058
	Temp. = 3.59°C		08–12	14	1.363	0.898	0.465
			12–16	69	0.564	0.452	0.112
			16–20	68	0.880	0.628	0.252
	R (fish) = 0.0216		20–24	43	1.248	0.891	0.357
	R (other) = 0.0118				$\bar{S} = 0.969$	$\bar{S} = 0.738$	$\bar{S} = 0.231$
					DR = 0.45	DR = 0.38	DR = 0.07
SE[a]	Winter	<40					
SE	Winter	≥40	24–03	13	2.679	2.662	0.018
			03–06	12	1.493	1.482	0.011
	Temp. = 2.64°C		06–09	7	1.438	1.405	0.033
			09–12	28	0.141	0.080	0.061
			12–15	25	1.302	1.259	0.043
	R (fish) = 0.0194		15–18	31	1.050	0.941	0.109
			18–21	11	1.325	1.305	0.021
	R (other) = 0.0106		21–24	10	0.912	0.715	0.197
					$\bar{S} = 1.293$	$\bar{S} = 1.231$	$\bar{S} = 0.062$
					DR = 0.59	DR = 0.57	DR = 0.02

[a] Insufficient data at one or more time periods.

Histopathology and the Pathogenesis of Embolism (Gas Bubble Disease) in Rainbow Trout (*Salmo gairdneri*)

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Rainbow trout (*Salmo gairdneri*) affected with gas bubble disease (GBD) were examined histologically to determine the pathogenesis of the early stages of gas emboli formation. Lesions preserved by a rapid fixation method were located in tissues associated with acid-secreting glands or with high metabolic requirements. Following the development of small gas emboli formed in the retinal chorio-capillaries, a progressive development of unilateral exophthalmia was detected. A lesion found in every treated moribund fish but never in controls was gas displacement of the blood from the afferent arteriole within the gill filaments. The exposure of fish to well water containing graded levels of atmospheric gases from 114 to 118% nitrogen and 103 to 110% oxygen saturation resulted in high mortality. However, fish held in nitrogen varying between 103 and 117% and oxygen from 50 to 94% saturation experienced insignificant mortality. We suggest that hatchery oxygen levels should be maintained below 100% if nitrogen supersaturation is present to reduce mortality from GBD.

Nous avons effectué un examen histologique de truites arc-en-ciel (*Salmo gairdneri*) souffrant d'embolie gazeuse afin de déterminer la pathogénèse des premiers stades de la formation des embolies. Les lésions, préservées par une méthode de fixation rapide, étaient situées dans des tissus associés aux glandes à sécrétion acide ou présentant des besoins métaboliques élevés. Suite à la formation de petites embolies gazeuses dans les choriocapillaires de la rétine, nous avons observé le développement progressif d'une exophthalmie unilatérale. Chez tous les poissons moribonds traités, mais chez aucun des poissons témoins, nous avons observé le déplacement du sang par le gaz dans l'artériole afférente, à l'intérieur des filaments branchiaux. L'exposition des poissons à de l'eau de puits contenant des teneurs mesurées en gaz atmosphériques, de 114 à 118 % d'azote et de 103 à 110 % d'oxygène, a provoqué une forte mortalité. Cependant, les poissons maintenus dans de l'azote à saturation de 103 à 117 % et dans l'oxygène à saturation de 50 à 94 % n'ont manifesté qu'une mortalité insignifiante. Nous proposons de maintenir, dans les piscicultures, la teneur en oxygène à moins de 100 % s'il y a sursaturation en azote, afin de réduire la mortalité due à l'embolie gazeuse.

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Problems with embolism in hatcheries are typically associated with water derived from springs that have naturally become supersaturated with atmospheric gases or from wells which have gas introduced by the pumping process. High mortality has occurred yearly in some Michigan State fish hatcheries. For example, in the newly constructed Harrietta State Fish Hatchery, personnel reported a 92% mortality in rainbow trout (*Salmo gairdneri*) juveniles in 1981. In 1982, over two thirds of the steelhead (*S. gairdneri*) fry and fingerlings died at Wolf Lake Hatchery, and in 1983, losses of steelhead and brown trout (*Salmo trutta*) were as high as 70%. Pendill's Creek National Fish Hatchery lost 1.3 million lake trout (*Salvelinus namaycush*) in 1983. The Marquette Fish Hatchery lost all their 2 million lake trout fingerlings in 1984. A clearer understanding of the pathogenesis of gas bubble disease (GBD) as it occurs in hatcheries might lead to better design for new hatcheries and other water-related construction projects.

Gross lesions in fish with GBD have been well described (Rucker 1972; Stroud and Nebeker 1976; Bouck 1980), but with a few exceptions (Pauley and Nakatani 1967; Nebeker et al. 1976; Bell et al. 1986), histologic descriptions defining the location and effects of gas emboli in the fish under conditions of supersaturation are lacking.

Our major objective was to determine if the early-phase emboli distribution was associated with specific tissue/organ types under variable, graded water supersaturation conditions similar to those found in high-mortality hatcheries.

Materials and Methods

Two trials were conducted using the same experimental design and apparatus. Fish used in trial 1 were rainbow trout fry, 9 wk old, with an average total length of 4.5 cm. They were reared in the Aquaculture Research Laboratory at Michigan

State University. For trial 2, the same size, age, and type of fish were obtained from the Baldwin Fish Farm, Baldwin, MI. Fish were fed twice a day with Biodiet Grower (Bioproducts, Inc., Astoria, OR). The fish were exposed to artificial light that simulated a natural photoperiod with a ratio of 14 light to 10 dark hours a day.

To create a higher than normal gas tension in the rearing trays, well water was supersaturated by pumping air into a vertical column through an air-diffusing stone. Pressurized air was pumped into the base of the 4 m high by 15 cm diameter water column.

The water column was contained in a (PVC) pipe, open on the upper end to allow excess gas to escape and fitted with an overflow system. Supersaturated water was released from the base of the column into the rearing trays placed 30 cm above one another. Each tray measured 38 × 50 × 12 cm and held 16.2 L. In trial 1, the flow rate was adjusted to produce a water renewal in the trays of 200 mL/min. The water flow was changed to 400 mL/min at day 46 of the 60-d test. In trial 2, the water flow in the trays was maintained at 400 mL/min during the course of the 20-d test to determine if the mortality in trial 1 was a result of alterations in individual gas pressures resulting from change in waterflow.

Each trial was designed with four treatments. The first three treatments (A, B, and C) had four replicates and the fourth treatment (D) had three replicates.

The desired level of supersaturation for each test tray was maintained by a water flow cascading system. The water was allowed to cascade in free-fall through 30 × 4 cm PVC pipes in transit from tray to tray. The splashing action allows the water contact with air at atmospheric pressure, therefore decreasing the level of supersaturation. In both trials, treatment A had the highest supersaturation levels. The gas saturation levels progressively decreased as the water flowed down the trays to treatments B and C. Treatment D, which had the same well water source, but did not undergo supersaturation, served as a reference or the true control. Specimens taken from reference trays were used as control samples.

The well water supply at the Aquaculture Laboratory has a nitrogen supersaturation of 105% which is similar to that in hatcheries where problems with GBD have been occurring. In the control group, treatment D, in which the water used did not undergo artificial supersaturation, the levels of nitrogen ranged from 104 to 106% saturation throughout the test. The total dissolved gas saturation and oxygen saturation were below 100%.

Fish in the 15 shallow test trays from the 2000-cohort holding tank were allocated to groups in a fashion similar to dealing a deck of cards. The first group of five fish netted from the holding tank was placed in the first test tray, the second group of fish in the second tank, etc. The sixteenth group of five fish was placed in the first tray, the seventeenth in the second tray, etc. This was continued until each test tray contained 50 fish.

Dissolved oxygen, total dissolved gas pressure, barometric pressure, water temperature, and water vapor pressure were measured weekly in every test tray. Dissolved oxygen was determined by the modified Winkler method utilizing a Hach's digital titrator (Loveland Co.). An electronic tensionometer (model 300C, Novatech Designs LTD, Victoria, B.C.) was used to determine supersaturation by measuring the total gas pressure (millimetres of mercury) of all dissolved gases in the water, called differential pressure (dP). The tensionometer also measures the local barometric pressure. Bunsen's solubility coefficient (B) for oxygen was obtained from tables in Weast (1980).

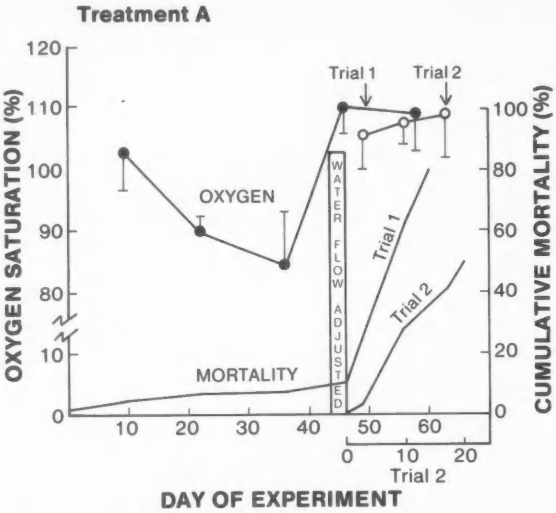


FIG. 1. Effect of oxygen on the rate of cumulative mortality (treatment A, Tables 1 and 2). Exposure of 3-mo-old rainbow trout to nitrogen supersaturation ranging from 114 to 118% produces significant mortality only if oxygen is also supersaturated. Upward water flow adjustment during trial 1 (box) from 200 to 400 mL/min resulted in both oxygen supersaturation and mortality in treatment A of trial 1 but neither in treatments B, C, and D (not shown in Fig. 1). In trial 1, oxygen saturation (solid circles) was elevated on day 46 to 60 and the cumulative mortality was accelerated during that period. Trial 2, a 20-d experiment, was designed to repeat the final 14 d of trial 1 with constant water flow to test if mortality was due to elevation of oxygen (open circles) or due to changing the water flow. The oxygen saturation standard error is shown as bars extending from those values.

The test well water had the following known chemical and physical characteristics: hardness (as $CaCO_3$), 330 mg/L; alkalinity (as $CaCO_3$), 260 mg/L; pH, 7.5–7.7; temperature, 12.0 ± 1.0°C.

Saturation levels of all gases were determined by computer using as a basic program the following formulae improved by Bouck (1982):

$$(1)\quad \text{Total dissolved gas saturation} = \frac{100(P + B)}{B}$$

$$(2)\quad \text{Oxygen \% saturation} = \frac{(\text{oxygen pressure in water})100}{(\text{oxygen pressure in air})}$$

$$= \frac{(P_{O_2})100}{(B - P_{H_2O})\,O_2\text{ mole fraction in dry air}}$$

$$= \frac{(P_{O_2})100}{(B - P_{H_2O})0.2095}$$

$$(3)\quad \text{Nitrogen \% saturation} = \frac{(P_{N_2})100}{(N_2\text{ mole fraction in dry air})(B - P_{H_2O})}$$

$$= \frac{(P_{N_2})100}{(0.78084)(B - P_{H_2O})}.$$

TABLE 1. Numbers of fish in exposure, mortality, and dissolved gas content of the experimental water at 12°C. Rainbow trout were exposed for 60 d to varying levels of dissolved gases (trial 1).

	% cumulative mortality (test day)[a]	Total dissolved gas in % saturation (mean ± *t*0.05 SE)	Nitrogen in % saturation (mean ± *t*0.05 SE)	Oxygen in % saturation (mean ± *t*0.05 SE)
Treatment A	5.0 (9)	113.7±0.9	116.9±1.5	102.6±6.2
(*n* = 200)	7.0 (22)	109.8±0.5	115.2±0.6	89.8±2.2
	8.5 (36)	110.2±1.2	117.2±0.8	84.5±8.4
	11.0 (46)	115.7±1.2	117.5±0.4	110.1±4.6
	63.5 (56)	115.6±1.2	117.8±0.4	108.7±5.4
Treatment B	0.0 (9)	103.2±1.1	108.0±0.1	85.3±5.4
(*n* = 200)	0.0 (22)	98.7±1.4	108.0±1.0	63.0±5.4
	0.0 (36)	98.3±2.4	108.3±2.4	60.5±7.9
	0.5 (46)	105.1±0.9	109.4±0.6	89.9±2.4
	0.5 (56)	105.1±1.0	109.9±0.3	87.7±3.4
Treatment C	0.0 (9)	98.4±1.0	104.5±0.2	75.3±2.9
(*n* = 200)	0.0 (22)	94.8±0.9	104.7±0.6	57.0±5.7
	0.5 (36)	94.1±0.7	105.6±2.0	50.2±9.9
	0.5 (46)	99.1±0.5	106.4±0.3	71.7±3.4
	0.5 (56)	99.2±0.5	106.9±0.4	70.1±4.1
Treatment D	0.0 (9)	97.6±2.7	104.9±3.1	70.2±1.2
(*n* = 150)	0.5 (22)	96.9±2.9	104.4±3.1	68.4±2.5
	0.5 (36)	94.7±3.6	106.5±3.9	49.9±2.5
	0.5 (46)	96.3±2.2	106.4±1.2	57.9±5.9
	0.5 (56)	96.2±2.1	106.5±0.9	57.3±7.2

[a] Total mortality after 60-d test for treatments A–D: 80.0, 0.5, 0.5, and 0.5%, respectively.

When fish with typical signs of GBD became moribund, they were collected from trial 1 for histological examination. Asymptomatic fish from the control trays were used for comparison. A recently developed technique utilizing heated Bouin's solution for rapid fixation of the tissues was used (Bell et al. 1986). During the test, 12 moribund fish were selected for histological study along with an equal number of control fish. At the end of 60 d of the experiment, the 422 survivors were examined and 104 were randomly selected for microscopical study. Representative gross and histologic lesions were photographed.

Each specimen was sampled so that whole fish, viewed with a dissecting microscope (macroscopic examination), as well as fixed multiple microscopic sections were surveyed. It was thus possible to make gross and microscopic comparisons. Lesions were categorized by making a blind comparison among treated and control fish. As gross lesions were encountered, additional sections of gills, liver, and kidney were made and examined.

Mortality curves (percent mortality versus time exposure) were plotted for each level of supersaturation. Mortality data were calculated to one half of one percent. Mortality rate among treatments was compared using chi-square tests. The Bonferroni chi-square test was used to compare each experimental group with the control group.

Results

Mortality

The exposure of fish to the highest supersaturation level, treatment A, resulted in a high morbidity and mortality, reaching 80% at the end of the 60-d test in trial 1 and 50% after the 20-d test in trial 2. In the latter trial, the exposure differed only in the constant water flow conditions and in the shorter duration of the exposure (Fig. 1). Signs consistent with GBD were found in moribund or recently dead fish.

In both trials, fish held in treatments B, C, and D experienced no mortality. The levels of nitrogen saturation in these treatments varied from 103 to 111%. It is emphasized that these test groups had oxygen levels below 100% varying from 50 to 94% saturation (Tables 1 and 2).

The onset of mortality, in trial 1, occurred in treatment A 2 d after exposure to 116.9 ± 0.7% nitrogen and 102.6 ± 6.2% oxygen saturation. Progressive mortality reached 6% in the first 10 d. Thereafter, the nitrogen levels remained constant at about 116%, but the oxygen levels diminished gradually to 84.5 ± 8.4%. There was insignificant mortality during the period from day 10 to day 46. On day 46 the water flow was changed from 200 to 400 mL/min. The increase in water flow increased the oxygen saturation level to 110.1 ± 4.6% in treatment A. Death began to occur rapidly at that point and continued, reaching 80% at the termination of the test. Mortality in trial 2 started after 2 d and constantly progressed until the end of the test.

Mortality rates were significantly different for the four treatments ($P = 0.001$; chi-square test). Using the Bonferroni chi-square test to compare mortality rates of each of treatments A, B, and C with treatment D, only treatment A was significantly different from treatment D ($P < 0.01$).

Because of the rapid growth of the fry and an initial water flow of 200 mL/min, there resulted a decrease of the level of oxygen in the water as the fish grew. This caused the oxygen tension to decrease to 5 mg/L in the lower trays. Since the system did not have an external oxygen supply, the hatchery-recommended minimum level of 6 mg/L oxygen was not maintained. Therefore, the water flow in trial 1 was increased on day 46 of the 60-day experiment. In trial 2, oxygen tension was maintained above 7 mg/L.

Coincident with the trial 1 water flow change, even while the

TABLE 2. Numbers of fish in exposure, mortality, and dissolved gas content of the experimental water at 12°C. Rainbow trout were exposed for 20 d to varying levels of dissolved gases (trial 2).

	% cumulative mortality (test day)[a]	Total dissolved gas in % saturation (mean ± t0.05 SE)	Nitrogen in % saturation (mean ± t0.05 SE)	Oxygen in % saturation (mean ± t0.05 SE)
Treatment A	2.5 (3)	111.6±0.7	113.5±1.5	105.1±5.7
(n = 150	28.0 (10)	113.0±0.5	114.6±0.2	108.1±4.0
	41.5 (17)	113.4±1.7	114.9±1.7	109.0±7.4
Treatment B	0.5 (3)	105.7±1.0	109.2±1.2	93.1±0.7
(n = 150)	0.5 (10)	105.8±0.7	109.2±1.0	93.5±7.4
	0.5 (17)	105.8±2.2	111.4±3.0	85.2±3.7
Treatment C	1.0 (3)	101.9±0.2	106.1±1.0	86.2±4.5
(n = 150)	1.0 (10)	101.8±1.0	107.4±2.7	81.1±7.9
	1.0 (17)	100.7±0.7	107.4±3.0	75.1±8.9
Treatment D	0.5 (3)	99.0±2.7	104.3±0.7	79.2±6.4
(n = 150)	0.5 (10)	100.0±1.7	104.3±0.2	83.5±8.9
	0.5 (17)	100.3±1.5	103.2±1.0	89.4±6.4

[a] Total mortality after 20-d test for treatments A–D: 50.0, 0.5, 1.0, and 0.5%, respectively.

nitrogen levels remained constant, the oxygen levels increased substantially in all treatment trays. These fortuitous adjustments in water flow resulted in the significant observation that oxygen and nitrogen levels each play a different role in determining the mortality at relatively low total dissolved gas saturation.

Even though the injected volume of air produced by a 0.5-hp motor at 1.4 atm of pressure was constant throughout the test, the oxygen level at day 36 of the test was significantly different ($P < 0.01$) from the level on day 46 of the test, after the adjustment of water flow. The levels of nitrogen and total dissolved gas saturation were not significantly different ($P > 0.01$), however. It is important to note that the elevated level of nitrogen combined with the increased oxygen levels could have raised supersaturation above the lethal threshold, and this was associated with extensive mortality. Because the percent saturations of nitrogen, oxygen, and total dissolved gases were not directly comparable as a result of variation within groups and between determinations, the threshold saturation level could not be calculated.

Pathology

The signs usually associated with GBD were rarely observed prior to the convulsive stage which occurs a few minutes before death. Signs noted in rainbow trout fry included loss of equilibrium, abnormal buoyancy, and aimless swimming as the convulsive stage commenced. Fish started swimming side- and belly-up with violent whirling movements interspersed with periods of inactivity followed by spasmodic convulsions (the moribund stage) leading to death. Fish frequently died with the mouth agape and the gills and operculum flared.

Gross pathology findings confirmed reports of Shirahata (1966), Rucker and Kangas (1974), and Stroud et al. (1975). The most common grossly detectable lesion was subcutaneous tissue emphysema elevating the skin (Fig. 2A). Another common lesion was emphysema of the caudal fin, dorsal fin, pectoral fins, and anal fins (Fig. 2B).

Gross lesions of GBD also included periorbital hemorrhages in some affected fish. Petechial hemorrhages were commonly observed in the gills and in the opercular and branchiostegal areas. Fish appeared to die most frequently during periods of stress such as feeding, gas monitoring, or any manipulation that increased muscular activity.

Exophthalmia

In 5 of 50 fish, in treatment A, there was unilateral exophthalmia (Fig. 3A), and one fish had bilateral exophthalmia. In the severe form, the globe of the eye was prolapsed outward from a point adjacent to the optic disk (Fig. 3B), resulting in a space forming between the capillary layer of the choroid and the pigmented epithelial layer of the retina. This space occupied up to one half the volume of the normal globe. No particular inflammatory lesion was associated with the prolapse.

The earliest histologic ocular lesion was an emphysematous space within a vessel of the choroid body of the eye (Fig. 3C). Another lesion, more advanced and presumably the precursor lesion to globe prolapse, was a separation of the retina from the choroid with an intact optic nerve. The dissecting separation resulted in formation of an oval tent of retinal tissue around the optic nerve. The separation occurred at the disk which served as the point of remaining attachment (Fig. 3D). In the normal eye there was an intimate association between the choroid gland, choroid layer, and the pigmented epithelium of the innermost retinal layers. In the affected eye, degenerative vacuolization of the optic nerve, musculature, adjacent connective tissue, and glandular tissue was occasionally evident.

Gill Lesions

A lesion found in every moribund treated fish was gas displacement of the blood from the afferent arteriole within the gill filaments. The lesion was never observed in controls. The afferent arteriole was identified by its anatomical association with the cartilaginous support of the gill arch. The efferent arteriole, which was not marked by gas-blood displacement, was identified by anatomical characteristics of the fleshy, broad end of the gill filament (Fig. 4A–4D). In addition, the ventral aorta, which supplies blood in branches into the afferent arterioles immediately after leaving the bulbus arteriosus of the heart, was occasionally recorded as also having a lesion of

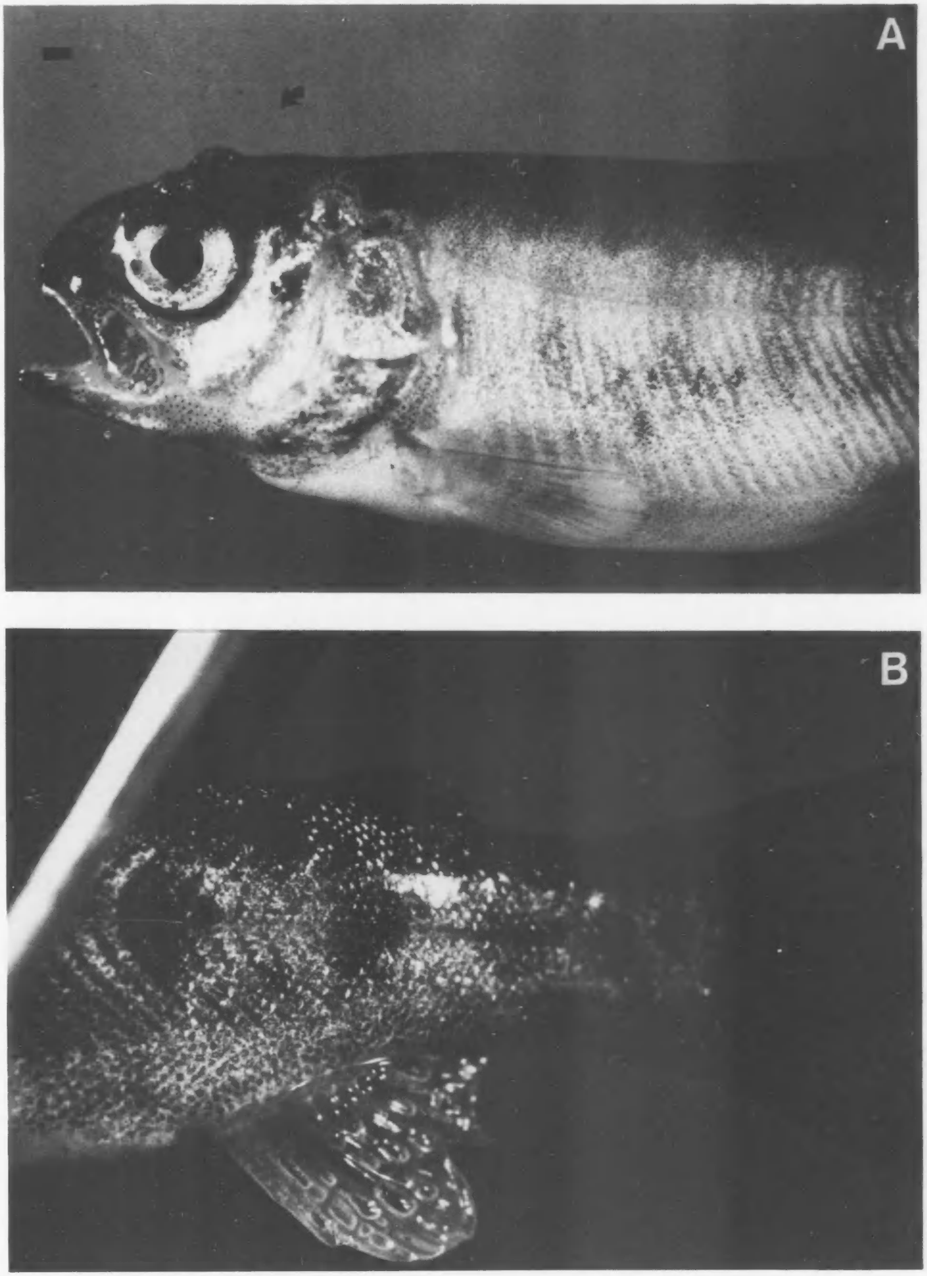


FIG. 2. Three-month-old rainbow trout fry exposed to supersaturation levels of 117% nitrogen and 110% oxygen. (A) Focal elevation of the epithelium over the caudal margin of the operculum and over the skull (bar = 1 mm); (B) emphysematous lesions of the anal fin (bar = 1 mm).

gas-blood displacement (Fig. 5B), while the control sections did not have such lesions (Fig. 5A). The findings suggested that the origin of gas was from the venous circulation with buildup in the afferent side of the gill arches.

In two fish, an emphysematous space was observed within the skull. The spaces surrounded the medulla oblongata and were continuous with semicircular canals. The lesion was visible on sagittal and on cross section in one fish. It may have been an artifact caused by an oblique sectioning of the auditory passages which gave the appearance of emphysematous lesions.

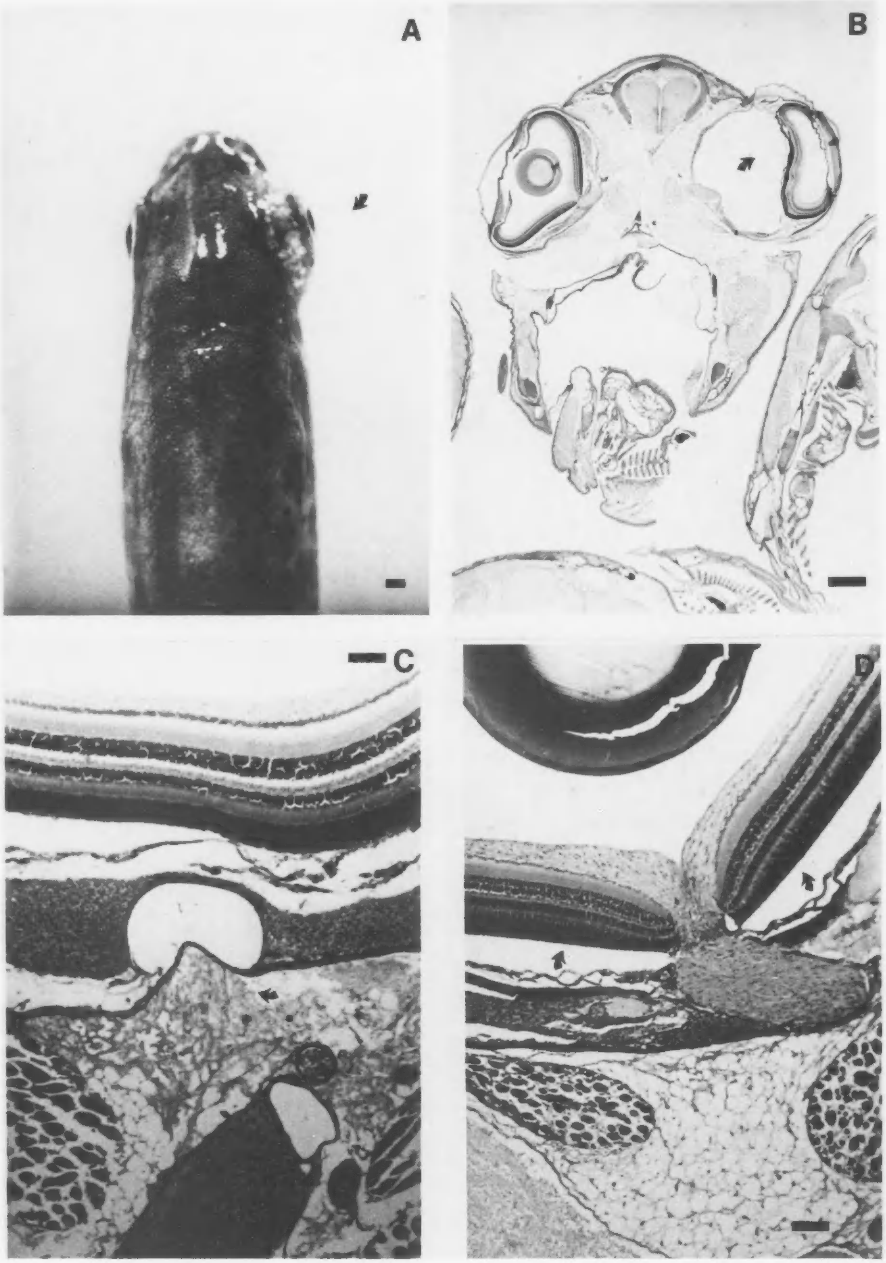


FIG. 3. Three-month-old rainbow trout fry exposed to supersaturation levels of 117% nitrogen and 110% oxygen. (A) Marked unilateral exophthalmia (bar = 1 mm); (B) macroscopic lesion of severe unilateral exophthalmia (bar = 0.5 mm); (C) lesion of the early exophthalmia manifested by the presence of an air space in a vessel of the choroid gland (bar = 50 μm); (D) precursor lesion to occular prolapse in GBD-induced exophthalmia (H & E stain) (bar = 50 μm).

Control sections revealed undilated semicircular canals containing an amorphous protein fluid.

Additional nonemphysematous lesions were observed in 70 individual fish. These lesions were randomly distributed among treated and control groups with no frequency association with a particular supersaturation exposure. They consisted of various gill lesions, extraordinary liver glycogen accumulation, fatty vacuolization of the liver, possible mineralization of kidney

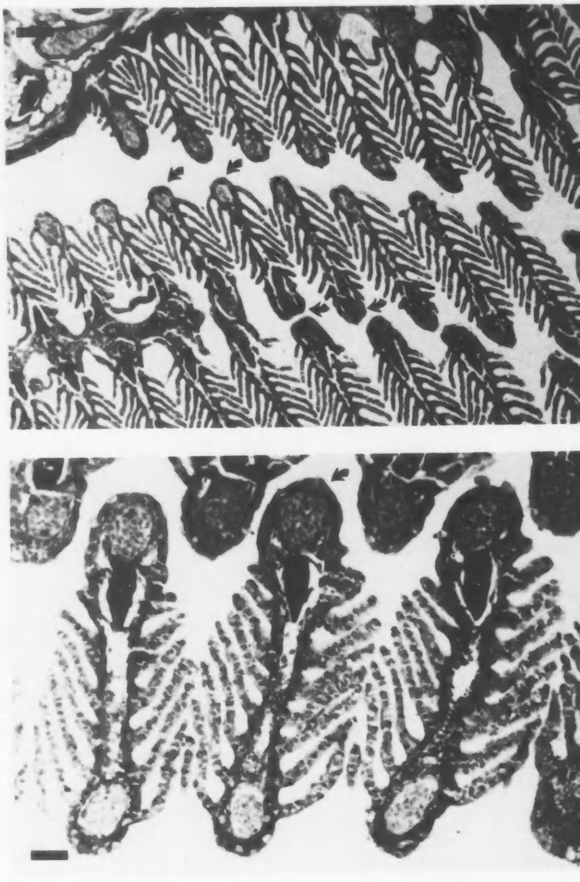
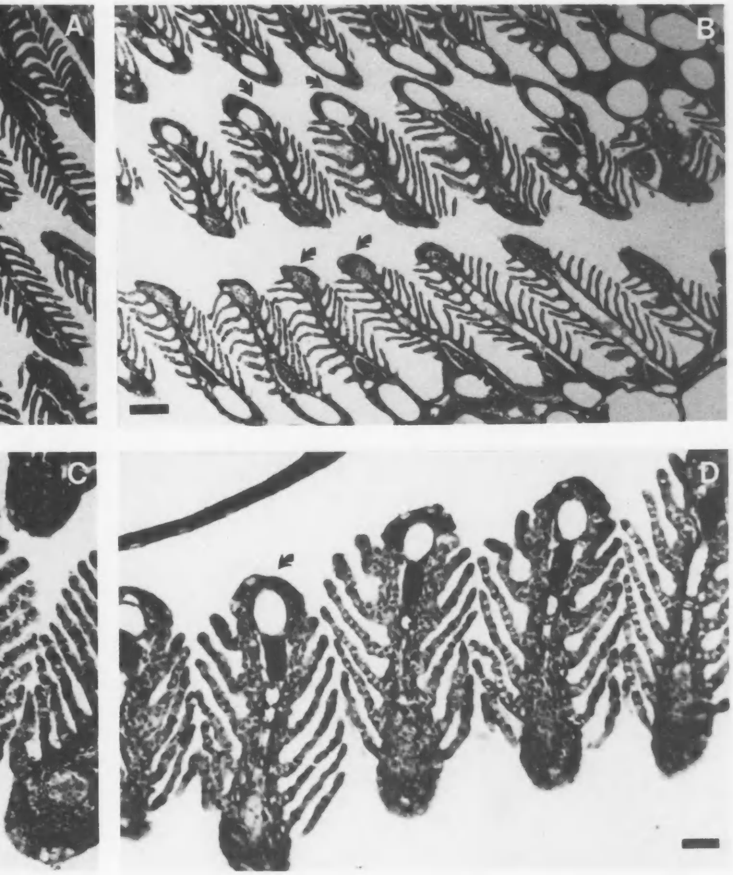


FIG. 4. Oblique sections of the gill filaments of 3-mo-old rainbow trout fr
support of the gill arch. The efferent arteriole is identified by anatomica
efferent arterioles of control group specimen (bar = 50 μm); (B) treated fi
content of the efferent arterioles (bar = 50 μm); (C) detail of the gill filam
(D) detail of the gill filaments of a treated specimen demonstrating gas



trout fry. The afferent arteriole is identified by its anatomical association with the cartilaginous
atomical characteristics of the fleshy, broad end of the gill filament. (A) Normal afferent and
eated fish indicating gas displacement of the blood from the afferent arterioles and normal blood
ill filaments of a control specimen showing the appearance of normal arterioles (bar = 20 μm);
g gas displacement of the blood from the afferent arterioles (H & E stain) (bar = 20 μm).

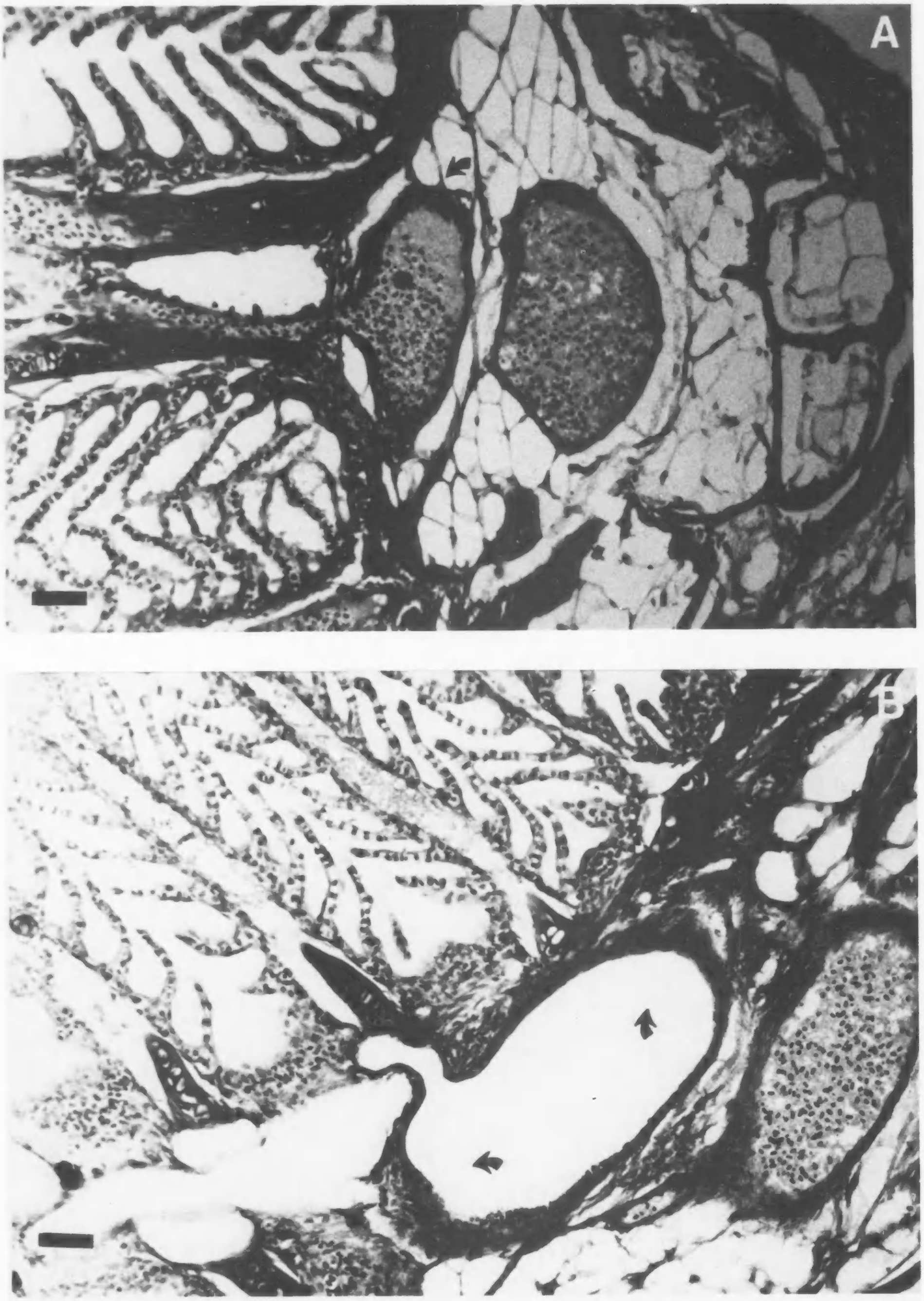


FIG. 5. Longitudinal sections of the gill arch of 3-mo-old rainbow trout fry. (A) Control specimen indicating the normal appearance of gill structure and ventral aortic vasculature without gas-blood embolization (bar = 50 μm); (B) treated specimen showing the ventral aorta with evidence for gas displacement of blood close to the origin of the afferent branchial arteries (H & E stain) (bar = 50 μm).

tubules, and suspected lymphoid hyperplasia of the thymus gland associated with gill alterations. Gill lesions, seen in 21 fish on gross and/or histological examination, consisted predominantly of epithelial hyperplasia. This change was segmental or nodular in two fish, but most often diffuse and associated with accumulation of mucus. Gill filament blunting and fusion was observed sporadically when sections of whole gill arches were sagittally cut, but not frequently when gills were cross sectioned.

Discussion

The significant mortality observed in these trials occurred only when both oxygen and nitrogen levels were above 100% saturation. The influence on mortality of altering the P_{O2} in the gas mixture is suggested by the findings in trial 1, since nitrogen as high as 117% was not lethal in the absence of oxygen supersaturation. The lethal level under these experimental conditions is confirmed in trial 2. Histological examinations revealed that the distribution of lesions is specific and is located in tissues associated with acid-secreting glands or with high metabolic requirements, such as choroid glands and gills.

Our histological observations confirm the gross lesions reported by some investigators who did not examine GBD lesions histologically and others who were unable to detect microscopic lesions when standard tissue fixation techniques were used. Signs of GBD were reported to have disappeared after death and the carcass was without diagnostic lesions (Bouck 1976). Marsh and Gorham (1905) described gas bubbles in the gill filaments as the most constant and significant macroscopic lesion. Nebeker et al. (1976) observed that when the gill arches of GBD-affected fish were cut, gas bubbles were released from the branchial arteries, indicating that gas emboli were present under pressure in the bloodstream flowing to the gills. They also observed gas emboli in the choroid vessels supplying the retina of the eye.

Attempting to explain the physiological processes that occur in fish dying of GBD, it is important to distinguish between the respiratory gases, oxygen and carbon dioxide, and nitrogen. Translation of the oxygen tension from water into the blood depends on blood flow, water flow over the gills, and hemoglobin affinity. The equilibrium between hemoglobin and oxygen is influenced predominantly by the P_{CO2}, the pH, and the body temperature. Fish hemoglobin is relatively sensitive to fluctuations of each of these factors when compared with the mammalian hemoglobins (Powers 1975).

Trout hemoglobin is especially sensitive to such changes, and therefore, the Root effect and the Bohr effect are prominent (Eddy 1971). The binding affinity of hemoglobin for oxygen thus varies with fluctuations of temperature and acidity (Powers 1975). In fish, the difference between the P_{CO2} of inspired and expired water is small but that of P_{O2} is great, since oxygen affinity for hemoglobin is high. Unlike mammals, fish monitor their blood oxygen, not blood carbon dioxide. Therefore, changes in environmental nitrogen, blood carbon dioxide, or total gas saturation in water which do not involve changes in oxygen are not directly conpensated by altered respiratory effort.

Nitrogen passively diffuses across the gill membrane and the tension in the blood should reflect that in the water. Nitrogen supersaturation of blood has not been demonstrated (DeJours et al. 1968).

A GBD pathogenesis hypothesis by Bell and Farrell (1972) is based on the assumption that violent oxygen unloading, characteristic of some fish hemoglobin under conditions in which blood is gas saturated, forms small oxygen emboli. These emboli build to form larger emboli under conditions of high total dissolved gas pressure.

Fish respond to excess oxygen levels by slowing respiratory and circulatory efforts even though their tissues may be accumulating lactic and carbonic acids. This metabolic process produces a decrease in the blood pH which is enhanced by the increase in the partial pressure of carbon dioxide. This process could precipitate a rapid oxygen unloading from red blood cells and would form the oxygen emboli. These minute emboli would tend to enlarge when released into a gas-supersaturated bloodstream. This process may occur even faster during physical stress, as in feeding or handling, since the increase in muscular activity can raise the partial pressure of carbon dioxide in venous blood from 2.5 to up to 8 mmHg (Holeton 1971a, 1971b; Randall 1975). Stroud and Nebeker (1976) observed that fish more frequently died of gas embolization shortly after physical stress was induced.

In this study, histopathologic lesions were found earliest in the gill arches, choroid gland of the eye, the skull, and the peripheral tissues. Since each of these anatomical sites is associated with acid secretion resulting in accelerated oxygen exchange, the lesion distribution supports the hypothesis that oxyhemoglobin may play a role in the initial generation of GBD emboli.

It was in construction of new hatcheries in 1980 that GBD was first recognized as a serious problem in the state of Michigan. Since well water is frequently supersaturated, a practice currently used in Michigan fish hatcheries to remedy nitrogen supersaturation is to increase aeration. However, in those situations, oxygen commonly increases above saturation level and the nitrogen excess is not completely bubbled out. Thus, the problem may become aggravated. High oxygen levels in the field have not been assessed with regard to GBD. It is suggested that when a case of GBD is reported, oxygen saturation be documented in addition to nitrogen and/or total dissolved gas saturation.

We endorse the stratagem of reducing oxygen saturation when nitrogen supersaturation is present to reduce mortality from GBD.

Acknowledgments

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Dietary Protein Requirements of Fishes — A Reassessment

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It is widely believed that fishes require more dietary protein than other vertebrates. Many aspects of fish physiology, nutrition, and trophic ecology have been interpreted within the context of this high protein requirement. Here, fishes are compared with terrestrial homeotherms in terms of (1) protein requirement for maintenance, (2) relative protein concentration in the diet required for maximum growth rate, (3) protein intake rate required for maximum growth rate, (4) efficiency of protein retention in growth, and (5) weight of growth achieved per weight of protein ingested. The two animal groups compared differ only in relative protein concentration in the diet required for maximum growth rate. This difference is explained in terms of homeotherms' greater requirement for energy and does not reflect absolute differences in protein requirement. The remaining measures of protein requirement suggest that fishes and terrestrial homeotherms are remarkably similar in their use of protein as a nutritional resource. Reinterpretation of the role of protein in fish physiology, nutrition, and trophic ecology is perhaps in order.

Il est généralement admis que les poissons ont besoin de plus de protéines dans leur alimentation que les autres vertébrés. De nombreux aspects de la physiologie, de la nutrition et de l'écologie trophique chez les poissons ont été interprétés en fonction de ce besoin. La présente étude compare les poissons à des homéothermes terrestres en ce qui concerne 1) les besoins en protéines pour le métabolisme, 2) la concentration relative en protéines des aliments nécessaire pour obtenir le taux de croissance maximum, 3) le taux d'absorption des protéines nécessaire pour obtenir le taux de croissance maximum, 4) l'efficacité de la rétention des protéines pour la croissance et 5) la croissance en poids, en fonction du poids des protéines ingérées. Les deux groupes d'animaux comparés différaient seulement par la concentration relative des protéines dans l'aliment qui était nécessaire pour obtenir le taux de croissance maximum. Cette différence s'explique par le fait que les homéothermes ont un plus grand besoin d'énergie, mais ne reflète pas de différences absolues dans les besoins en protéines. Les autres mesures des besoins en protéines semblent indiquer que les poissons et les homéothermes terrestres sont remarquablement semblables en ce qui concerne leur utilisation de protéines comme ressource nutritive. Il est peut-être nécessaire de réinterpréter le rôle des protéines dans la physiologie, la nutrition et l'écologie trophique des poissons.

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A first principle of fish nutrition is that fish as a group are fundamentally different from other vertebrate animals in that they require more dietary protein (Cowey and Sargent 1979; Cowey and Luquet 1983; Tacon and Cowey 1985; Wilson and Halver 1986). Mammals and birds typically achieve maximum growth rate on diets containing 12–25% protein, whereas fish require diets with 35–55% protein to reach maximum growth rate. In general, fish nutritionists are of the opinion that the efficiency of protein utilization by fishes is lower than that of other animals (Rumsey 1981).

The roles of fishes in both wild and captive populations are frequently interpreted within the context of this high protein requirement. Pandian and Vivekanandan (1985) pointed out that most fish species feed as predators and concluded that fish have adapted over evolutionary time to utilization of, and dependence on, this protein-rich diet. They suggested that most species are confined to a carnivorous feeding habit by the need to meet protein requirements, and question whether fishes can actually subsist on low-protein foods such as algae, vascular plant matter, or detritus without a supplement of animal prey in the diet. Because protein is usually the most expensive component of aquaculture diets, many aquaculturists consider dependence of fish on high-protein diets to be an unfortunate limitation to the efficiency of fish production (Pandian and Vivekanandan 1985; Knights 1985).

Although widely held, these views are not consistent with the observation that many fishes that specialize as detritivores and herbivores are extraordinarily successful in supporting a large population biomass on very low protein diets (Dabrowski 1982; Bowen 1984a, 1984b, 1987). To resolve the apparent conflict, in this paper I compare published data on use of dietary protein by fishes and by other vertebrate animals for maintenance and growth. The results do not support the view that fishes in general require more dietary protein than other vertebrate animals.

Methods

Most published data on protein requirements of animals are from studies intended to help maximize growth performance in agriculture or aquaculture. Measures of whole-animal performance that may be compared to evaluate a broad, across-taxa generalization such as that addressed here are often not reported by authors and must be calculated from two or more data presented in tabular form. Over 100 publications were reviewed to identify those that contained data necessary for calculation of the measures of protein requirement and growth performance discussed below. In each case, criteria used to select appropriate data for specific measures of protein requirement are specified.

Many nutritionists expect carnivores to have significantly greater requirements for protein than herbivores, detritivores, or omnivores. Studies of mammals compiled by MacDonald et al. (1984) indicate that the domestic cat (*Felis domesticus*), a carnivore, requires more protein than man (*Homo sapiens*), the rat (*Rattus norviticus*), or the dog (*Canis familiaris*), which are omnivores. Inasmuch as this generalization applies to fishes, the most meaningful comparisons will be those made between fishes and other animals within a single trophic group. However, studies conducted to date appear to lack sufficient precision to allow detection of such a difference. In a recent review, Pandian and Vivekanandan (1985) found that "The mean protein requirement of omnivorous, herbivorous and detritivorous fishes is equal (about 49% of dry food ingested) to that of carnivores." Data presented below are taken largely from a different group of studies than those consulted by Pandian and Vivekanandan, but are consistent with this conclusion (Table 1). Thus, no distinction between carnivorous and noncarnivorous fishes has been made here.

In this work I distinguish between *relative* and *absolute* measures of dietary protein requirement. *Webster's Ninth New Collegiate Dictionary* defines relative (adj) used in this sense as "not absolute or independent" (Merrium-Webster Inc. 1985). The percentage of protein in the total diet weight required for maximum growth rate is a relative measure. It is a ratio numerator whose value depends directly on the quantities of other components in the diet that make up the denominator. If 2 kg of cellulose is added to 25 kg of diet mixture, the relative concentration of protein decreases although the absolute quantity of protein in the mixture remains the same. In contrast, protein ingestion rate at maximum growth (milligrams ingested per gram of fish, Tacon and Cowey 1985) is an absolute measure of protein requirement that is quantified without reference to other components of the diet.

As noted by Wilson (1985) and Wilson and Halver (1986), fish do not have a requirement for protein per se, but rather require amino acids that are usually obtained from the diet by digestion of protein. However, the term "protein requirement" is used here for consistency with the extant literature.

Statistical analyses were performed by the STATISTIX program for microcomputers (NH Analytical Software, St. Paul, MN) according to the procedures of Snedecor and Cochran (1980).

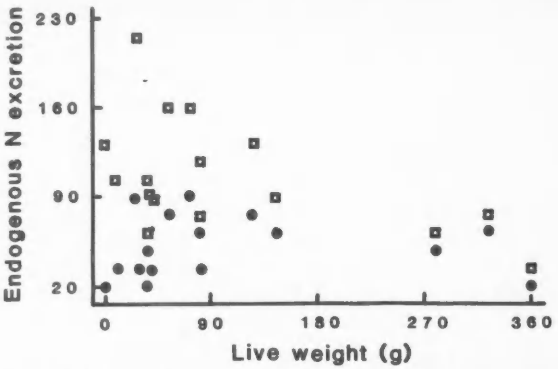

FIG. 1. Weight-specific endogenous nitrogen excretion estimates from 16 studies of fish. Units are mg N·kg fish weight^{-1}·d^{-1} (open squares) and mg N · kg$^{-0.75}$ fish weight $^{-1}$·d^{-1} (solid circles).

Protein Requirements for Maintenance

Under conditions allowing no growth (no weight change), all animals have a relatively low, yet absolutely necessary requirement for dietary protein to replace proteins lost in maintenance and metabolism (Brody 1945). This requirement can be estimated experimentally as the nitrogen excreted by a fasting animal (endogenous nitrogen excretion, ENE) (Brett and Groves 1979). Given the measurements of ENE that have been made for fishes and other vertebrate animals, do these establish a higher protein maintenance requirement in fishes?

Brett and Groves (1979, table V) summarized ENE estimates for fishes in units of milligrams of nitrogen excreted per gram of fish per day. Of the 24 estimates listed, exact weights or narrow weight ranges are given for 16 of them. Estimates expressed in these units are strongly influenced by fish weight (Fig. 1, weight–ENE correlation = -0.54, $n = 16$, $p < 0.05$). In studies of mammalian and avian physiology, it is conventional to scale ENE estimates to weight raised to the exponent 0.75 (Robbins 1983). This eliminates body size as a factor and thus makes it possible to compare ENE estimates for animals of different sizes. Similarly, calculating ENE as milligrams of nitrogen excreted per kilogram $^{-0.75}$ of fish weight per day removes the effect of size on ENE estimates for fishes (Fig. 1, ENE–weight correlation = -0.07, $n = 16$, $p \gg 0.05$). It is particularly important that the effect of size be removed in the present case because, within the group of animals for which ENE estimates are available, the fishes are smaller than all but one of the mammals (rat). Although temperatures at which these measurements were made ranged from 12 to 26°C, there was no apparent effect of temperature on ENE (temperature – scaled ENE correlation = 0.21, $n = 16$, $p \ll 0.05$; temperature – unscaled ENE correlation = 0.11, $n = 16$, $p \ll 0.05$).

Estimates of ENE for fishes average 51.0 mg N excreted·kg$^{-0.75}$ fish weight^{-1}·d^{-1} ($n = 16$, SD = 21.9), while ENE estimates for 13 mammals including 10 wild species (Robbins 1983) and rats, dogs, and humans (Munro 1964) average 100.2 mg N excreted·kg$^{-0.75}$ fish weight^{-1}·d^{-1} ($n = 13$, SD = 39.5). The median for mammals is significantly higher than the median for fishes (Wilcoxon's signed rank test, $p = 0.002$). This result is not dependent on the choice of 0.75 as the weight scaling factor for fishes: values of 0.80 and 0.65 also give statistically

TABLE 1. Growth performance of young fishes and other young vertebrates fed diets with protein concentrations that promote the highest growth rates observed under the experimental conditions used.

Animal	Specific growth rate[a]	% protein in diet	Protein intake at maximum growth[b]	Protein retention efficiency[c]	Protein growth efficiency[d]	Food conversion efficiency[e]	Authority
			Fishes				
Chanos chanos	4.91	39.1	40	21	1.22	0.51	Lim et al. 1979
Cyprinus carpio	3.20	41.5	16	30	2.15	1.12	Takeuchi et al. 1979
Cyprinus carpio	2.61	40.6	11	40	2.90	0.83	Ogino et al. 1976
Cyprinus carpio	4.83	39.0	17	48	2.85	1.11	Murai et al. 1986
Ictalurus punctatus	2.63	32.0	10	40	3.44	1.10	Garling and Wilson 1976
Micropterous dolomieu	2.85	45.3	21	27	1.36	0.58	Anderson et al. 1981
Micropterous salmoides	2.36	39.9	16	31	1.48	0.65	Anderson et al. 1981
Morone saxatilis	2.56	57.7	19	25	1.70	0.96	Millikin 1983
Pleuronectes platessa	0.93	50.0	13	23	1.50	0.75	Cowey et al. 1972
Salvelinus alpinus	2.03	36.0	10	33	2.03	0.74	Jobling and Wandsvik 1983
Salmo gairdneri	2.66	53.0	10	40	2.82	0.67	Ogino et al. 1976
Sarotherodon mossambicus	3.84	42.0	25	25	1.68	0.96	Jauncy 1982
	2.68	40.0	16	37	2.95	1.18	Jackson and Capper 1982
Sciaenops ocellatus	3.95	44.0	32	32	1.90	0.81	Daniels and Robinson 1986
Scopthalonus maximus	3.78	80.0	19	29	1.99	1.19	Bromley 1980
Tilapia nilotica	4.73	28.0	17	40	2.50	0.69	De Silva and Perera 1985
Tilapia nilotica	6.62	35.0	53	26	1.25	0.56	Santiago et al. 1982
Tilapia nilotica × *T. aureus*	1.20	35.0	7	31	1.71	0.59	Viola and Zohar 1984
Median (fishes)	2.765	40.3	16.5	31	1.945	0.78	
			Other vertebrates				
Calf	0.40	12.8	3	24	1.51	0.22	NRC 1984
Chicken	4.21	20.0	21	44	2.08	0.42	NRC 1971
Chicken	6.07	20.0	12	37	2.48	0.43	Leeson & Summers 1980
Chicken	8.46	25.0	?	38	2.35	0.53	Buamah & Singsen 1975
Lamb	3.00	25.4	16	30	1.85	0.20	NRC 1985
Lamb	0.75	13.5	1.2	24	1.24	0.14	Ames and Brink 1977
Swine	1.68	12.0	6	25	2.46	0.30	Kropf et al. 1959
White rat	1.89	22.0	24	28	0.79	0.17	Hartsook et al. 1973
Median (other vertebrates)	2.445	20.0	12	29	1.965	0.26	

Rank sum test for differences between central values; fish vs. other vertebrates; two-tailed probabilities that central values are the same

	0.638	0.0001	0.173	0.638	0.563	0.0001	

[a] Specific growth rate = 100 $\log_e (\text{weight}_t \cdot \text{weight}_0^{-1}) \cdot t^{-1}$ (Brett 1979).

[b] mg protein ingested · g body weight^{-1} · d^{-1}.

[c] 100 · g protein retained · g protein ingested^{-1}.

[d] g growth · g protein ingested^{-1}.

[e] g growth · g diet ingested^{-1}.

significant differences. Direct comparison with ENE values for birds is more difficult because avian excreta is mixed in the cloaca with a small but imprecisely known quantity of metabolic fecal nitrogen, nitrogen in sloughed intestinal cells, and unrecovered digestive enzymes which are not considered in estimates for mammals or fish. Observations compiled by Robbins (1983) for six species of wild birds averaged 96 mg N excreted · kg$^{-0.75}$ fish weight $^{-1}$ · d^{-1}. Given differences in experimental details and the limited number of species examined to date, it is perhaps premature to conclude that fishes require less dietary protein for maintenance than other animals. However, the available evidence clearly cannot be used to support the conclusion that fishes require more protein than other animals for maintenance.

Protein Requirements for Growth

Expressed as a percentage of diet dry weight, the concentration of protein in the diet that produces maximum growth is the most frequently used measure of protein requirement for growth of fish (Pandian and Vivekanandan 1985). Requirements summarized in Table 1 are from 18 studies of fish and 8 studies of nonfish vertebrates in which a series of diets containing different protein levels were fed to young animals ad libitum, or at rates close to those expected under ad libitum feeding. The requirement value is the protein concentration above which no significant increase in growth was found under the experimental conditions used. Values in Table 1 from National Research Council summaries are for optimal diets which appear to be similar to those required for maximum growth. A comparison of relative protein concentration in the diet required for maximum growth of fishes and nonfish vertebrates echoes many previous studies cited above in showing that a much higher relative concentration is required in the diets of fishes.

This observation must be interpreted with caution. Although there is unquestionably a difference between fishes and other nonfish vertebrates, this difference may be related only indirectly to protein requirements. As with all relative measures, there are two equally likely interpretations that may be made. The relative protein concentration required by fishes may be higher because (1) fishes need more protein for growth (numerator relatively greater) or (2) fishes require less of some other diet component (denominator relatively smaller). Thus, the relative concentration of protein in the diet required for maximum growth does not, on its own, establish a high protein requirement in fishes.

In view of the shortcomings of relative composition as a measure, Tacon and Cowey (1985) proposed measurement of protein requirements in terms of protein intake rate (milligrams per gram of body weight per day) required to support maximum growth. Results summarized in Table 1 indicate that fishes and nonfish vertebrates are not significantly different in this regard. While this approach has the advantage that it quantifies protein requirement in absolute terms, considerable variation is introduced by the fact that fish of different physiological ages held at different temperatures differ dramatically in their scope for growth and thus in the rate at which they can convert protein into growth (Brett 1979). Protein requirements expressed in these terms may differ because the animals studied use protein differently, they have different physiological growth potentials, they are held under different conditions of temperature or photoperiod, or due to some combination of these variables. For studies of fishes listed in Table 1, protein intake rate is the most variable of the four measures of growth performance, and most of this variation is explained by the protein intake –specific growth rate correlation ($R = 0.802$, $n = 18$, $p < 0.01$). Thus, factors other than the intrinsic requirement of fishes for protein contribute substantial variation to measurement of the protein intake rate required for maximum growth and limit its value.

Efficiency of Protein Utilization in Growth

Two ratios have been widely used since 1910 to assess the efficiency of protein utilization in growth (Brody 1945; Knights 1985). These describe the proportion of ingested protein that is retained in growth (protein retention efficiency, PRE) and the weight of growth achieved per weight of protein ingested (protein growth efficiency, PGE) (Table 1). PRE estimates are affected by rates of protein and energy intake and, at low rates of protein intake, by the amino acid composition of the diet (sometimes called "protein quality") (Miller and Payne 1961; Nijkamp et al. 1974; Boorman 1980). Thus, ony data from growth trials in which an effort was made to adjust both protein and energy intake to provide maximum growth rate under the experimental conditions used were included in Table 1. Both PRE and PGE estimates for mammals and birds are affected by the animal's physiological age (Brody 1945). As an animal matures, its scope for growth per unit weight decreases while the proportion of its diet that is committed to maintenance increases. A diet that provides just enough protein for maximum growth of a young animal will provide excessive protein for an older animal with the consequence that the extra protein will be catabolized and PRE will be reduced. In White Leghorn chickens (*Gallus gallus*) fed a diet containing 20% protein, PRE is 43.8% for a 370-g animal, but drops to 26.8% for a 1130-g animal (NRC 1971). Similarly, Leeson and Summers (1980) found that PRE in chickens declined steadily from a maximum of 59.8% for chicks during the first week after hatching to 26.1% after 10 wk of growth. Although physiological age has a similar affect on scope for growth in fishes (Brett 1979), Huisman et al. (1979) compared PRE for carp (*Cyprinus carpio*) across a broad size range (3–488 g) and found that "Retention of protein was in the same range for all fish sizes used [in the study]." To increase the likelihood of detecting less efficient protein utilization for growth by fishes compared with nonfish vertebrates, results included in Table 1 are for young animals that have, in most cases, achieved less than 50% of their maximum size. These data for both PRE and PGE unambiguously fail to support the generality that fish require more protein than terrestrial homeotherms for growth. Similar conclusions were reported by Cho and Kaushik (1985, p. 112) and Wilson and Halver (1986, p. 239).

Indirect Inferences of an Absolute High Protein Requirement in Fishes

The evidence reviewed above includes all types of measures of protein requirement based on whole-animal performance that are known to the author. The results do not support the widely held view that fishes require more dietary protein in any absolute sense. In contrast with the results of whole-animal performance studies, several authors have extrapolated from studies of carbohydrate digestion and carbohydrate and amino acid metabolism at the molecular level to infer that fish do require more protein than other animals in an absolute sense (reviews by Rumsey 1981; Cowey and Luquet 1983). The major points raised in this detailed work are reviewed below.

Carbohydrate as a Source of Metabolic Energy

There is an ongoing controversy concerning the ability of fish to use carbohydrate for metabolic energy. Several authors have reasoned that if fish cannot use carbohydrate and do not for any reason use enough lipid for metabolic energy, then they necessarily depend on protein as a source of metabolic energy.

Many fish do not digest processed raw starch efficiently. Observations originally reported by Phillips et al. (1948, cited in Bergot 1979b) and repeated by others that salmonids digest as little as 20% of raw starch in practical diets have been taken as evidence that fish must rely on protein as an energy source. However, cooked or "gelatinized" starch can be digested by rainbow trout (*Salmo gairdneri*) with an efficiency of 87–90% (Bergot and Breque 1983). Spannhof and Plantikow (1983) showed that raw starch binds amylase such that 70% of the hydrolytic activity is lost but that cooked starch causes little reduction in hydrolysis. Thus it may be that the low digestibility of commercially dehydrated starch is an artifiact of diet preparation. Simpler carbohydrates such as glucose and sucrose are readily digested by salmonids and other fishes (Bergot 1979a, 1979b; Pieper and Pfeffer 1980a, 1980b). The data published to date do not lead to the unambiguous conclusion that fish in general cannot digest or assimilate adequate carbohydrate to meet energy requirements that cannot be met by metabolism of lipids.

Results from many studies have indicated that fishes have a limited ability to catabolize carbohydrate (Cowey and Luquet 1983; Tacon and Cowey 1985), but others show that fish can utilize energy in glucose, sucrose, and gelantinized starch as or more efficiently than protein energy to support metabolism (Bergot 1979a, 1979b; Pieper and Pfeffer 1980a, 1980b; Kaushik and de Olivia Teles 1985). Similarly, opinion is divided as to whether fishes utilize stored glycogen as a source of metabolic energy during periods of starvation (Murat et al. 1981). Much work remains to be done before these apparent discrepancies can be resolved.

To keep expectations in perspective, it is important to recognize that fish would not benefit from high levels of dietary carbohydrate that are beneficial to homeotherms because they do not require a lot of "metabolic fodder" for temperature maintenance. In work cited above, levels of carbohydrate were raised to 30% without a decline in growth rate. If carbohydrate levels had been further increased at the expense of protein, we might predict that growth would be slowed, not by an inability to catabolize carbohydrate, but by an unfavorable protein/energy balance in the diet. These experimental diets typically contained 10–18% lipid and 30–45% protein in addition to 30% carbohydrate. With an energy density of approximately 21 $kJ \cdot g^{-1}$, these diets contained about 22 mg protein·kJ food $energy^{-1}$, or just slightly below the protein/energy ratio expected to provide maximum growth (Bowen 1979, 1984b). Thus, a further increase in the carbohydrate content of the diet would be expected to deprive the fish of protein needed for maintenance and growth.

Regulation of Amino Acid Catabolism

In a number of detailed studies, the effects of diet composition on tissue levels of enzymes characteristic of gluconeogensis, glycolysis, and amino acid catabolism were investigated. Rainbow trout were used as the experimental model in most of this work. In general, high-protein diets resulted in higher levels of enzyme activity in the gluconeogenic pathway and lower levels of enzyme activity in the glycolytic pathway, relative to high-carbohydrate diets (Walton 1986). In contrast, activity levels of enzymes involved in amino acid catabolism were significantly less responsive to diet composition. From this observation, several authors have reasoned that fish lack the ability to conserve amino acids in low-protein diets and thus, in the absolute sense, must ingest more protein than other animals to meet their nutritional requirements (Rumsey 1981; Cowey and Luquet 1983). For several reasons, this inference may be premature. Tissue concentrations of amino ascids are typically well below levels required to saturate the enzymes that would catabolize them, and thus, levels of amino acids, not levels of catabolic enzymes, are likely to provide proximate regulation of amino acid catabolism (Walton 1986). More fundamentally, the rate of reaction measured in vitro may bear little relation to the rate of reaction in vivo. The many mechanisms that affect distributions of enzyme and substrate within organelles, within cells, and within tissues all offer opportunities for regulation of amino acid catabolism in vivo that go undetected by in vitro assay. Indeed, more recent research by Buddington, Cowey, and Walton indicates that trout are able to adapt the rates of amino acid catabolism in order to conserve amino acids for growth (R. K. Buddington, Department of Physiology, University of California at Los Angeles, pers. comm.). Finally, if fishes are unable to regulate amino acid catabolism, we would expect them to be far less efficient than other animals in use of protein from high-protein diets. Data presented in Table 1 do not show that this is the case.

In summary, the results of detailed studies of carbohydrate digestion and carbohydrate and amino acid metabolism are in some cases consistent and in other cases inconsistent with the hypothesis that fishes require more dietary protein than other vertebrate animals. Such findings provide insight into differences between animal groups and suggest explanations for phenomena at higher levels of organization. However, we are far from an understanding of animal physiology and nutrition that is sufficiently complete as to permit confident prediction of whole-animal performance from studies at the molecular level, and thus, these detailed observations cannot provide evaluation of such a hypothesis.

Conclusion

Two circumstances appear to have led to the conclusion that fishes require more protein. First, the requirement for diets *relatively* high in protein has been taken as an indication of a higher *absolute* requirement. Data summarized above indicate that the absolute difference in requirements of fishes and homeothermous vertebrates is in their requirement for energy, not protein. This point was recognized by previous authors (e.g. Rumsey 1981) but has not been integrated into the general perspective on protein requirements of fishes and, thus, the relative measure continues to be widely misinterpreted. Despite their inherent limitations, relative measures that can be used to describe diet composition are needed for day-to-day practical applications. To make the relationship between protein and energy requirements more clear, nutritionists working with animals other than fishes have used protein, the less variable quantity, as the denominator in such relative measures as "kJ metabolizable energy·mg^{-1} protein" (Sell et al. 1985), rather than "mg protein·kJ metabolizable energy" used by many fish nutritionists (Bowen 1984b).

Second, the majority of studies of digestive physiology and nutrition have dealt with exclusively carnivorous fishes, but the results often have been taken as indicative of fishes of all trophic types. At the molecular level, both homeotherms and poikilotherms show adaptation to use of the dominant macromolecules in their diets (Karasov et al. 1985), and thus, an abundance of particular enzymes for amino acid catabolism in carnivorous fishes fed high-protein diets can be expected. As noted by Rumsey (1981), Tacon and Cowey (1985), and others, similar adaptations have been found in carnivorous mammals. As comparative data become available for omnivorous, herbivorous, and detritivorous species, the significance of adaptations in digestion and metabolism for across-taxa comparisons will become more readily interpreted.

In conclusion, the view that the trophic role of fishes is severely constrained by an exceptionally high requirement for dietary protein cannot be supported by the available evidence. Many herbivorous and detritivorous fishes with low-protein diets are extraordinarily successful in establishing large populations which comprise more than half the fish biomass in some aquatic ecosystems (Bowen 1984a, 1987). Why are fishes such as *Sarotherodon mossambicus* and *Prochilodus platensis* the only vertebrate animals able to achieve rapid population growth using very low protein detritus as their only food resource (Bowen et al. 1984)? The answer appears to lie not in unique nutritional requirements, but in other aspects of fishes' trophic behavior such as food selection and adaptations in digestion.

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A Simulation of the Cape Breton Snow Crab, *Chionoecetes opilio*, Fishery for Testing the Robustness of the Leslie Method

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The principal tool for evaluating biomass and, hence, exploitation rate for Atlantic snow crab, *Chionoecetes opilio*, has been the Leslie method which is based on commercial catch rate and cumulative catch through the fishing season. The method assumes a closed, homogeneous stock. However, a snow crab stock is often not closed because recruitment can occur during the fishing season as crabs molt into legal size. Also, stocks are not homogeneous and spatial heterogeneity causes fishermen to combine fishing and searching activities. A dynamic simulation of fishing/searching on a heterogeneous sessile stock with within-season recruitment was developed, based on the Cape Breton fishery, to assess the robustness of the Leslie method. In the modelled system, the violations of the assumptions caused by recruitment were shown not to be serious; but those due to the fishing/searching on a heterogeneous stock caused the Leslie method to underestimate initial biomass. Furthermore, the concepts of initial biomass and exploitation rate are seen to be inadequate for heterogeneous, recruiting stocks.

Le principal instrument d'évaluation de la biomasse, et donc du taux d'exploitation, du crabe des neiges (*Chionoecetes opilio*), est la méthode Leslie, qui est basée sur le taux de capture de la pêche commerciale et sur le cumul des captures pendant toute la saison de pêche. Cette méthode se fonde sur un stock qui serait clos et homogène. Cependant, un stock de crabe des neiges n'est généralement pas clos, car le recrutement peut se produire pendant la saison de pêche, à mesure que les crabes atteignent la taille légale par une mue. De plus, les stocks ne sont pas homogènes, et l'hétérogénéité spatiale fait que les pêcheurs doivent combiner activités de pêche et recherche. Nous avons mis au point une simulation dynamique de la pêche et de la recherche sur un stock sessile hétérogène avec recrutement en cours de saison, fondée sur la pêcherie du Cap-Breton, afin d'évaluer la solidité de la méthode Leslie. Dans le système modélisé, les manquements aux hypothèses causés par le recrutement se sont révélés peu graves; par contre, ceux qui étaient dus à la pêche et à la recherche sur un stock hétérogène ont fait que la méthode Leslie sous-estime la biomasse initiale. En outre, les concepts de biomasse initiale et de taux d'exploitation semblent inadéquats pour les stocks hétérogènes avec recrutement.

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The management of the Atlantic snow crab *Chionoecetes opilio*, fishery off Cape Breton, Nova Scotia, depends on estimates of stock abundance which are based on fleet performance (Elner and Bailey 1986). The performance of vessels, in turn, depends on the interrelated problems of discovering local concentrations of crabs and, subsequently, exploiting them. As the crabs are immobile when compared with the fleet, once a concentration has been discovered it can be exploited until the fishing effort is removed. The existence of, and the behavior of searching and fishing for, local concentrations is not taken into account in the model (Leslie method) which is used to estimate stock size and exploitation rate. By simulation, we shall estimate the robustness of the Leslie method to the principal violations of its underlying assumptions. Similar considerations have recently been presented (Allen and McGlade 1986) for the groundfish fisheries of the Scotian Shelf. Our work differs from theirs in that the system being described is composed of a single species and single-fleet fishery and that the results are being applied to a specific management model.

In many models of fisheries, it is assumed that as significant numbers of animals are removed from a closed stock, fishing success will be reduced. Implicit in this approach is that the fishing success is proportional to the stock abundance, where catchability (q) is the proportionality constant. Ricker (1975) reviewed the origins of using fishing success as a stock assessment technique.

An application of the relationship between catch-per-unit-effort (CPUE) and stock abundance was introduced by Leslie and Davis (1939) and amended by Braaten (1969). The Leslie methodology involves regressing a measure of fishing success (or any other index of abundance) against cumulative captures through a fishing season. The intercept of the regression line on the cumulative catch axis is an estimate of the initial stock size and the slope is an estimate of the catchability. The only requisite data are unaged catch and effort data; however, the approach is founded on the assumption of a closed, homogeneous stock. The estimated initial stock size is often used to calculate the exploitation ratio which is the total captures divided by the initial stock size.

Given the relative simplicity of the Leslie method, its modest data requirements, and that the biology of the species need not be well known, the technique has enjoyed wide usage in data-poor fisheries. In recent years, applications of the technique have been used to estimate the numbers of adult Northern pike, *Esox lucius*, in a large lake (Peterson et al. 1980) and to explore the dynamics of the California dungeness crab, *Cancer magister*, fisheries (Gotshall 1978; Methot and Botsford 1982). The Leslie method appears especially applicable to crab where traps provide an easily monitored effort unit. Biological advice for the Atlantic snow crab fishery in general and the Cape Breton stocks in particular has been based largely on Leslie analysis of fishing success gleaned from fishermen's logbooks (Bailey 1978; Elner 1982a, 1982b; Elner and Robichaud 1983; Taylor and O'Keefe 1981). Petersen estimates of snow crab stock size from mark–recapture studies have provided independent corroboration of Leslie estimates (Bailey 1978; Elner and Robichaud 1981). There has been concern, however, that since assumptions of the Leslie method, as identified above, are frequently not met, erroneous estimates of biomass and exploitation rate may have been generated.

The concern with the importance of failure to meet the strict assumptions has prompted our study. The magnitude of the effects of fishing a heterogeneous stock on the fishing success pattern cannot be determined either by logbooks or research surveys; the former lack suitable precision and the latter are impracticable due to the requirements to finely define the spatial and temporal dynamics of the resource. Therefore, in order to gain insight into the potential magnitude of the effects caused by the heterogeneity, a simulation incorporating fleet behavior was developed. The purpose of our model is to simulate the essential dynamics of the distribution of the resource and fishery. From the results of the simulation, we can evaluate the robustness of the Leslie method to relaxation of the assumptions. Our exercise was not intented to develop a complex analogue of the biology of the crab or the myriad psycho-economic determinants of fishing fleet behavior. Rather, we explored the problem by isolating and directly probing only the principal factors that affect fishing success. Thus, our model represents a novel approach to elucidating an otherwise intractable problem. We conclude that the Leslie method is surprisingly robust to an influx of animals during the season. However, failure to meet the homogeneous stock assumption may result in pronounced bias in the estimation of initial stock size.

Methods

Description of Fishery

The snow crab grounds off northwestern Cape Breton were open to 14 and 27 vessels in 1978 and 1979, respectively. Regulations limit the number of traps per vessel to 30 and restrict fishing to an approximate 2-mo (July–September) season. Management imposed a catch quota of 1406 t (52.1 t/vessel × 27 fishing vessels) in 1979 in an attempt to control fishing effort. As a condition of licence, snow crab fishermen are required to maintain logbooks detailing daily catch weight, effort (in terms of number of trap hauls) fishing location, and trap soak duration. The catch and effort data are lumped into 3-d totals which are 2/wk, as fishing does not take place on Sundays. Data from logbooks, together with information from at-sea and in-port sampling of commercial catches, have been used to provide annual assessments of commercial biomass, population structure, and exploitation rates. The duration of the recruitment pulse, and to some degree its magnitude, may be inferred from sampling landings for the incidence of soft-shelled, newly molted crabs (Elner 1982b). The geographical heterogeneity of the stock is reflected by changes in trap location (Fig. 1) where the fleet is searching for high-density "pockets" of snow crab yielding a high catch rate. The degree and partial cause of movement is estimated from an analysis of those logbooks wherein the vessel captains indicated a shift in position of their traps. This information was not required, and we are indebted to those captains who took the extra effort to include such details.

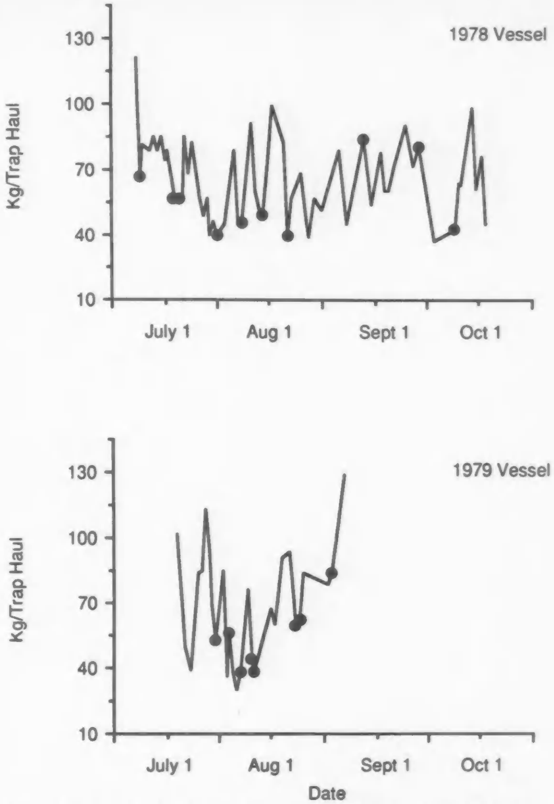

FIG. 1. Selected logbook data from vessels in 1978 and 1979 fisheries. Solid circles denote days in which a portion of the traps was moved.

Description of Models

The two principal assumptions under consideration will be modelled independently. In the first case, we consider recruitment into a homogeneously distributed stock and in the second, a heterogeneously distributed stock without recruitment. Either of these two cases can produce the plateau-type fishing success pattern seen in real fishing data (Fig. 2).

A recruitment pulse into a homogeneously distributed stock which results in a plateau followed by a declining fishing

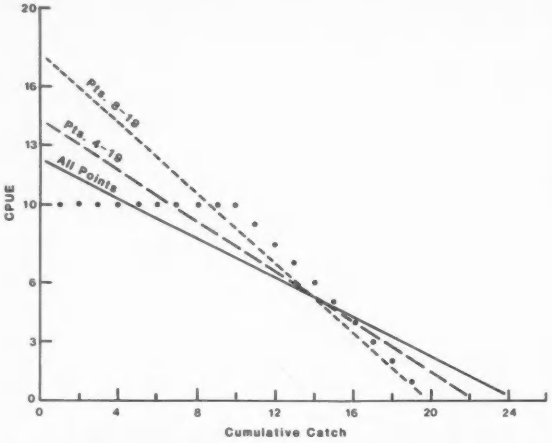

FIG. 2. Prototype of crab fishery CPUE versus cumulative catch data to show the influence of the breadth of the data window used for the Leslie analysis.

success trend (Fig. 2) can be modelled by partitioning the data series into two phases about the inflection. In this idealized case, removals during the initial plateau phase are exactly balanced by recruitment. In the declining phase the situation is that of a closed homogeneous stock into which recruitment has ceased and the Leslie assumptions are now met. The accumulated catch from the initial phase may be added to the Leslie estimate for the second phase to produce an estimate of the total available biomass for the season. This describes the prototype we shall investigate by simulation.

A computer model was developed to simulate the essential characteristics from the crab fishery data including spatially heterogeneous stock distribution and a mobile fleet in the absence of recruitment. The commercial data on which the model is based are from the 1978 and 1979 northwestern Cape Breton fishery and include catch, number of traps hauled, and number of vessels fishing. The unit of effort used in the simulation is 100 trap hauls with a standard soak time of 3 d, measures believed by the authors to more closely correspond to fishing mortality than vessel days at sea, a frequently utilized unit of effort (NAFO 1983). Trap hauls are also the units of effort used in the Leslie analysis of the actual fishery data.

To simulate the spatial heterogeneity of the resource, 16 subareas are defined: 10 at a low level of abundance, 4 at a medium level, and 2 at a high level (Fig. 3). An initial fishable biomass of 1400 t is distributed over these subareas, and no migration of the stock takes place. Also, there is no natural mortality applied, and the catchability is constant throughout the fishing season.

At the start of the simulated season, vessels are assigned randomly to subareas with a probability proportional to the crab abundance in each. Thus, the best areas were fished most heavily at the beginning of the season. The *temporal* pattern of total effort over all areas throughout the season is derived from the actual fisheries data (logbooks); the *spatial* distribution of the effort is determined by the fishing success. Generally, a vessel will remain fishing in the subarea it last fished. If fishing success falls beneath 60% of the average catch rate for the rest of the fleet in any given period, the vessel will stop fishing in its subarea and randomly choose a new one. Thus, success at fishing is defined in terms of the fleet as a whole and not on an absolute level. Once the decision to move to another subarea has been reached, the vessel is penalized 30% of the period's catch to account for the fishing lost in moving and resetting traps. The mechanism which determines in which subarea the vessel will be placed for its next fishing attempt is based on the relative abundance in the remaining subareas. An abundance level which is 84% of the mean level defines acceptable subareas for that time in the season. The vessel will be randomly placed in one of the acceptable subareas. The simulated fishery is controlled by two parameters: one that defines the lowest catch rate that a vessel will tolerate before searching for a new location, and a second which defines which grounds are sufficiently abundant so as to represent a desirable new location. If none of the new locations is "desirable," then the vessel will be randomly assigned to a new location with a probability which is proportional to the location's abundance. The model was developed in this manner to reflect fishermen's knowledge of the grounds and of what other members of the fleet are doing.

Six replicate simulations were performed for each year. The simulated data are analyzed using the Leslie method as is done for the commercial data in the following section.

Our model fishermen's behavior of searching or fishing is determined by the individual's catch rate as compared with the average for the fleet. It may be summarized as, "if fishing is poor, try another spot." During scrutiny of logbooks prior to Leslie analysis it was occasionally observed that fishermen would include comments about moving and resetting traps. Five logbooks from 1978 and nine from 1979 were felt to contain sufficient detail about searching/fishing that a summary analysis would be possible. The summary analysis compared catch rates before and after moving. The comparisons were made with the fleet average up to the given date as well as the individual's average from the first of the season.

Leslie Method

The basis for the Leslie method is the assumed linear relationship between CPUE (C/E) and average biomass ($\underline{B}$) in each unit period:

$$(1) \quad C/E = q\underline{B}$$

where q is the catchability. If one also assumes that the stock is closed, that is there is no net natural mortality, recruitment, or migration, then the only change in biomass is via captures (C), and thus:

$$(2) \quad C/E = qB_0D - qC$$

where B_0 is the initial biomass. Such is the basis for the Leslie regression and under the assumptions the relationship must be linear.

There is some difficulty in applying this method directly to commercial crab data (Fig. 3) which have the bilinear form. A prototype of the situation in the crab fishery has been shown in Fig. 2 wherein the CPUE is constant for the first 10 periods and then a constant slope for the remainder. Regardless of the underlying cause of such a bilinear form, which is frequently seen in crab fishery data, the three regression lines are seen to be dependent on the breadth of data in the window for analysis. Although the assumption of a closed stock is fairly well met in terms of mortality and migration, it is clearly violated if recruitment occurs during the season, as is often the case. The proportionality of the CPUE and stock abundance (i.e. that q is

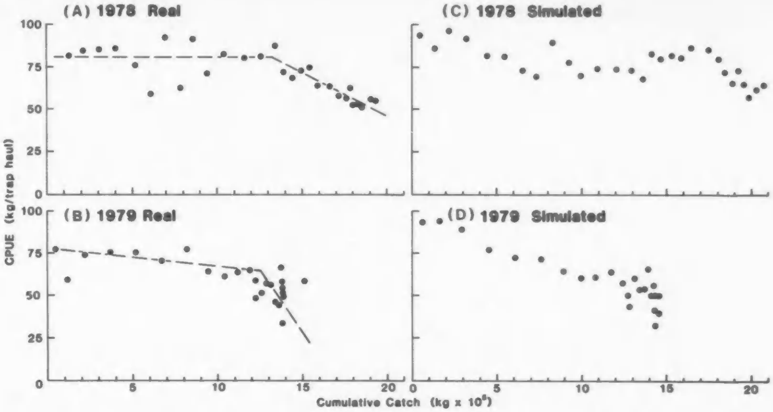

FIG. 3. Comparison of mean CPUE against cumulative catch data from logbooks of the snow crab fishing fleet off northwestern Cape Breton Island in (A) 1978 and (B) 1979 against data patterns generated from a simulation of the same fishery in (C) 1978 and (D) 1979. Broken lines have been added by eye to emphasize bilinear pattern in real data.

constant) should be a reasonable assumption unless the traps are saturating because of high abundance or long soak times. Because of the large traps employed and soak time of less than 3 d, it is assumed that trap saturation is not a significant factor. Traditionally, the plateau on the left-hand portion of Fig. 2 has been explained as the recruitment of snow crab approximately matching the withdrawals due to the fishery, and the common practice is not to include this portion in the regression. We shall show that fleet movement on a heterogeneous resource could also contribute to the formation of such a plateau.

Results

Total available biomass is the sum of the Leslie estimate (B_0) and the catch (C_0) which precedes the data window used in the Leslie regression. Estimates of available biomass from the prototypic data (Fig. 2) decreases as the portion of the data used in generating the Leslie estimate is progressively narrowed. The estimates of available biomass are at the intercepts of the regression lines on the x-axis, and when the data are partitioned about the time inflection the most accurate estimate is achieved (given a homogeneous stock with removals by the fishery being balanced by recruitment). We shall define this method as the accumulated catch – Leslie method (AC-L).

Both fishery and simulated data (Fig. 3) are analyzed using the Leslie method. In addition to considering the entire data set, analyses are done for abbreviated data sets starting part way through the season, as was described above. Table 1 presents these results. The total estimates of initial stock size (B) are the sum of the Leslie estimate (B_0) plus the sum of the catch preceding the inflection point (C_0). This approach, as previously mentioned, is appropriate for recruitment into a homogeneous stock.

As seen from Fig. 3, the actual fishing success data for 1978 show an extended plateau followed by a protracted declining phase. The estimates of total stock abundance (estimate from Leslie phase plus accumulated catch) decline monotonically as the Leslie phase is progressively constricted (Table 1). The simulated data from 1978 also demonstrated plateau and declining phases, even though no recruitment is included. The estimates of total stock abundance also decline, except for the final trial, with progressive constriction of the Leslie phase. However, the estimates are more similar to one another than those generated from the actual fishing data. Although the coefficients of variation (CV) are low from the simulated data, compared with the fishery data, the estimates of total stock abundance are approximately half the true value of 4200 t.

TABLE 1. Estimates of stock abundance (t) (B_0 = Leslie estimate of biomass; C_0 = accumulated catch preceding the initial point used in the Leslie analysis; B = initial stock size; CV = coefficient of variation[a]).

	Start–end of data	Fishery data				Simulated data[b]			
Trial	window	B_0	C_0	B	CV	B_0	C_0	B	CV[a]
1978									
1	1–29	5500	0	5570	0.11	2476	0	2476	0.03
2	5–29	4760	360	5120	0.13	1915	366	2281	0.02
3	10–29	2710	830	3540	0.08	1306	869	2175	0.03
4	15–29	1640	1370	3010	0.09	1115	1235	2350	0.06
1979									
1	1–24	4250	0	4250	0.12	2532	0	2532	0.05
2	5–24	2520	440	2960	0.11	1589	516	2105	0.03
3	10–24	1300	1080	2380	0.22	645	1165	1810	0.06
4	15–24	810	1280	2090	0.35	334	1339	1673	0.11

[a] Coefficient of variation is estimated as half the lower confidence limit at the 5% level divided by the expected value.

[b] True values of simulated initial biomass (B) are 4200 t for both 1978 and 1979.

The 1979 fishery data show a plateau phase, as for 1978, but the data thereafter do not demonstrate such a clear decline. Again, the estimates of total stock abundance decline monotonically with constriction of data for the Leslie phase; the estimate for Trial 4 is less than half of that for Trial 1 (Table 1). The estimates from the simulated data steadily decline but less steeply then those based on actual data. As for 1978, the true value of initial abundance in the simulation, 4200 t, is

TABLE 2. Summary of trap relocations and catch rates for 1978 and 1979 from a sample of logbooks.

	1978	1979
No. of vessels in subsample	5	9
Total no. of days fished	237	255
Total no. of relocations	60	75
Relocations when vessel's catch rate was lower than the fleet average	34	44
Relocations when vessel's catch rate was lower than the average to date	36	50
Change in catch rate after relocation	+4%	+ 7%

considerably above any of the estimates. The coefficient of variation appears markedly higher in Trial 4.

The summary analysis of the available data (Table 2) shows that there is a weak tendency to move when the catch rate is lower than the fleet average (34 moves out of 60 moves in 1978 and 44 out of 75 in 1979 were initiated when the individual rate was beneath the fleet average from the first of the season to the date in question). When the catch rate on the day before moving was compared with the individual average performance up to that rate, it appeared to be a slightly stronger indicator: 36 moves out of 60 moves and 50 out of 75. The catch rate on the day after the move was on average 4% higher in 1978 and 7% higher in 1979 than the rate before moving.

Discussion

The simulation was undertaken to assess the importance of the divergences between the formal assumptions of the Leslie method with regard to assessments on the Cape Breton snow crab fishery and, by inference, similar fisheries in which the effort is quite mobile and the resource relatively sedentary. The two principal violations of the assumptions are spatial heterogeneity of the stock, with the associated search/fish pattern of exploitation, and the recruitment to the fishable biomass during the season. Either of these phenomena could cause the plateau shape seen on the plots of CPUE versus cumulative catch. Discrimination between these two divergences using catch and effort data alone is impossible. However, in the case of Cape Breton snow crab, the incidence of soft-shelled, newly molted crabs is monitored and can be used to estimate recruitment, through growth, into the commercial size range. Also, through logbook analysis it is known that the fishermen do move traps about (Fig. 1) and experience rapidly changing catch rates.

The complex reality on the fishing grounds would comprise both actual recruitment and a heterogenous stock. We have examined the situation by creating two complementary models of the effects of fishing: (1) a homogeneous stock into which there is recruitment and 2) a heterogenous stock. While neither model is entirely realistic, each represents an extreme situation. The degree to which either model is appropriate depends on the fishery in question. Given a fishery dominated by recruitment as opposed to heterogeneity-induced effects, in the initial periods the technique of partitioning the data about the inflection point will give accurate results. On the other hand, if the spatial heterogeneity is the principal factor, serious underestimates of available biomass will be generated by data partitioning and Leslie analysis.

The two principal distinctions between the 1978 and 1979 simulated data sets are a more protracted recruitment period in 1978 and the absence of quota control. The protracted recruitment would act to extend the plateau, lowering the slope, and thus increasing the estimate of stock size.

An approximate factor of 2 is seen between the simple Leslie estimate (Trial 1) and the AC-L estimate when the inflection point is assumed to be late in the season. If one believes that the fishing success pattern is the result of recruitment, then the low AC-L estimate is appropriate; conversely, if spatial heterogenous effects are felt to be dominating, then the AC-L estimate will be an underestimate.

The relatively simple model used to depict the fishing of heterogenous grounds had 16 homogeneous subareas. The vessel movements and fishing among subareas were controlled by two parameters. The first parameter defines the lower limit of an acceptable catch rate which would cause the vessel to seek new grounds. The lower limit is a relative level in that the individual vessel catch rate is compared with the average performance of the fleet as a whole. The second parameter defines the minimum level of abundance a subarea would have to have to attract the vessel for the next period. The simple decisions of when to quit and where to go essentially mimic a feature of the Lotka–Volterra predator–prey description (Hassell 1978) for each subarea. When catches are good in a subarea, more vessels are attracted to it; the subarea is then depleted and the vessels (predators) leave.

The heterogenous model did not produce plateaus which were as protracted as those seen in the actual fisheries data. However, this is of little consequence, as our model was not developed to reproduce the actual data but rather to explore the potential biases of spatial effects. We are not suggesting that the plateaus are entirely due to spatial effects, but rather that they are one of several contributing factors.

The pattern of fishing success from the modelled heterogenous stock is seen to behave like recruitment into a homogeneous stock. Recruitment, as it is usually defined, denotes the addition of biomass into a fishery. This may be the result of crabs of a fishable size moving into the area of exploitation or the result of crabs in the area growing and, thus, becoming fishable. In heterogeneous stock situations, new crabs were also being made available for exploitation; but instead of the crabs moving to the fishing fleet, the fleet moves to the previously unexploited crabs. When we discuss recruitment, in the context of this paper, we mean the influx which balances removals during the plateau phase. We do not advocate the AC-L method as being appropriate for all possible recruitment patterns; our conclusions are restricted to the situation where we see an initial plateau followed by a declining phase in fishing success.

We have demonstrated that the fraction of the total area of the resource actually fished affects the Leslie estimate, in the case of an effectively sessile heterogenous resource. Similarly, in an analysis of mark–recapture data by the Petersen method (Ricker 1975) the area over which marked animals are distributed must correspond to the area subsequently fished. For example, if fishing occurred largely outside the mark–release area, recaptures would be too low and resultant population estimates too high. Thus, in studies where both Leslie and Petersen methods are used (Bailey 1978; Elner and Robichaud 1981, 1983), care must be taken in defining the various mark–release and fishing areas; otherwise, it is inappropriate to directly compare estimates.

We have adopted the term "total available biomass," which is the sum of recruitment and the initial biomass from the Leslie phase of the data. This concept is a more complete description for a homogeneous stock with recruitment than initial biomass alone. Conceptualization of the biomass available for fishing is more difficult in the spatially heterogenous case. During any given instant of the simulation there may be some subareas that are not being fished. Thus, the biomass being fished may well be less than the total of the 16 subareas. The amount available for fishing could be thought of as a total in all 16 subareas or as a total only in those subareas being fished. Because fishing success is derived only from those subareas being fished at any one time, estimates based on such data will underestimate the total. Thus, for clarity, we use the term total available biomass, which denotes the biomass available in all areas.

As well as biomass available for fishing, the concept of exploitation ratio needs to be reconsidered in light of our results. The classical definition of total catch divided by initial stock size is inadequate to deal with either recruitment or spatial heterogeneity. One could have very low initial stock and heavy recruitment and fishing, producing an exploitation ratio greater than 1. More distressingly, in the absence of recruitment, if the resource were distributed in a string of good holes which were exploited serially, the CPUE would not fall during the season, suggesting an infinite initial biomass and an exploitation rate of zero when the entire stock is removed. These admittedly extreme examples imply that the concept of exploitation ratio cannot be directly generalized outside the framework of the strict closed, spatially homogeneous model.

Our underlying data and the initial stock size estimate (B) have a temporal resolution of one unit period, 3 d, representing a temporal average. However, with a heterogeneously distributed stock, a spatial averaging is also required. Unfortunately, this average is over the grounds where the fleet is fishing, not over the entire potential fishable area. Hence, if richer grounds are found during the season, the catch rate will increase and affect the Leslie regression. If this increase occurs early in the data window, it will tend to increase the magnitude of the negative slope of the regression; and, conversely, it will decrease the estimated slope if it occurs late in the window. The effect may be further compounded by management-imposed restrictions, such as catch quotas, which may stop fishermen from searching later in the season. Such reduced searching late in the season would reduce the fished area and appear as an increase in q, as defined by the slope of the regression. This effect is noted when the efforts from logbook data from the 1978 and 1979 snow crab fishing seasons are compared. Thus, we contend that the simple nature of catchability (q) precludes its usage in spatially heterogeneous stock situations.

The amount of movement is seen to be a less critical factor than the type or quality of movement. However, two extreme cases of amount of movement are of interest. If the vessels do not move at all, the biomass will be estimated for the portion of the grounds fished. On the other hand, if their movement is totally random, the biomass for the entire area will be estimated. Intermediate amounts of random movement will result with biomass being estimated within these extrema. More serious than the degree of random movement is a concerted movement of vessels. If the vessels were to move as a group late in the season and find better grounds, extremely high or even negative biomass estimates could result. The simulations had deterministic criteria for search/fishing with a random initial distribution of effort, and the results were relatively insensitive to small (<10%) changes to the parameters controlling movement. The effects were of a similar magnitude to that due to the initial distribution. Given the high proportion of variability (apparently random) in the data from the fishery, the robustness of the AC-L method should not be compromised. This amount of movement cannot be explained in terms of economic reward, as Allen and McGlade (1986) suggested for "highliners," and is unexplained by the data available.

The low catch rate at the end of the 1978 fishing season is not seen at the beginning of the 1979 season. One explanation for this is that snow crab are slowing diffusing back into their preferred habitats on the fishing grounds. The diffusion rate is slower than the rate of removal during the fishing season, and its effects are evident only after a period without fishing activity, approximately 9 mo in the case of the Cape Breton snow crab fishery. Theoretical studies on diffusive systems (e.g. see Dubois 1975; Mimura and Murray 1978) demonstrate that patchiness of resource can be expected even in a homogeneous environment when the diffusion of the prey is small compared with that of the predator, as is the case for this fishery. Because the environment is not homogeneous, the tendency for patchiness or heterogeneity would be further enhanced by snow crabs diffusing into preferred habitats. Given such a scenario, we would expect that resource distribution would display predictable patterns from year to year. The importance of the distribution of the resource on the behavior of the fishermen and the causes of the distribution are topics intended for future study.

The brief analysis of those logbooks which had movement information suggests that catch rate is not the sole determinant of whether a fisherman searches or fishes. The small impovement, 4–7%, observed on new grounds suggests that the fishermen cannot predict where good sites are likely to be. Also, this small improvement would tend to reduce the importance of catch rate as a cause for relocating traps. It is neither possible, nor the purpose of this study, to illuminate the myriad reasons that may cause cessation of fishing or searching. However, such a study would be of obvious value if more detailed simulations were required.

In conclusion, we believe that the Leslie analysis can be an effective tool outside the strict confines of the closed, spatially homogeneous stock stipulation. The pattern observed in the snow crab fishery may be due to recruitment, heterogeneity, and/or fishermen's behavior. If a stock which shows the plateau fishing success pattern is felt to be affected by recruitment into homogeneous fishing grounds, then the AC-L method would be expected to yield unbiased results whose quality is dependent on the quality of the underlying data. Conversely, if such a pattern were caused by the movement of vessels exploiting a spatially heterogeneous stock, even high-quality data would result in serious underestimation of the total available biomass. Thus, accurate discrimination of these two factors is critical. In the case of the Cape Breton snow crab fishery for 1978 and 1979, recruitment is believed to have made a real contribution to the plateau (evidence from soft-shell data (Bailey 1978) and subsequent annual assessments of the fishery (Elner and Robichaud 1981)). Thus, the estimates generated were probably reliable. However, that this simple method is robust in some situations does not obviate the need for further refining the model or developing more sophisticated assessment techniques utilizing a better understanding of both the resource and the fishermen.

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Measurement of Egg Production Rate of *Calanus finmarchicus* from Preserved Samples

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Runge, J. A. 1987. Measurement of egg production rate of *Calanus finmarchicus* from preserved samples. Can. J. Fish. Aquat. Sci. 44: 2009–2012.

A method for determining *Calanus* egg production rates from preserved, net-tow samples is proposed. In the sea off Nova Scotia, in situ egg production rates (eggs per female per day) of *Calanus finmarchicus* are significantly related to an index of gonadal development in preserved females. This relationship could be used in combination with data on female abundance to estimate daily production of eggs in the water column. The method is illustrated with data from a transect across Browns Bank.

Une méthode de détermination des taux de production d'oeufs chez *Calanus* à partir d'échantillons formolés est proposée. Au large des côtes de la Nouvelle-Écosse, les taux de production d'oeufs *in situ* (oeufs par femelle par jour) de *Calanus finmarchicus* sont significativement correlés à un index du développement gonadique chez des femelles conservées. Combinée à des données sur l'abondance des femelles, cette relation pourrait être utilisée pour estimer la production quotidienne d'oeufs dans la colonne d'eau. Les données recueillies lors d'une étude effectuée dans la région du banc Browns servent à illustrer cette méthode.

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Variability in primary production cycles might affect year-class strength of fish by influencing the magnitude and distribution of food available to early life-history stages (Cushing 1982). In the North Atlantic Ocean, a key intermediary link is *Calanus finmarchicus*, whose eggs and naupliar stages are the primary prey of the earliest larval stages of cod, haddock, and other fish species of commercial importance (e.g. Kane 1984). Knowledge of temporal and spatial patterns of *Calanus* egg production would be useful for testing hypotheses about the importance of the linkage between plankton productivity and fish recruitment.

Because *Calanus* spawns eggs directly into the sea, present techniques for measuring egg production rates have problems that hamper their routine use. Immediate, postcapture observations of egg laying on ship (e.g. Runge 1985a) require handling of live animals and are labour intensive. Estimates of egg production from counts of egg density in the water column and data on egg development times (e.g. Checkley 1980) require sampling with fine-mesh nets and may not be accurate because of potentially high egg mortality rates (Peterson 1986). In this note, I outline a method for estimating *Calanus* egg production rates from preserved samples that can be collected routinely at sea. The basis of the method is a significant relationship between the in situ egg production rate and an index of gonadal development in preserved females.

Methods

Egg production rates of *C. finmarchicus* were measured at 17 stations in the sea off southwest Nova Scotia in June 1982 and May 1983 (Runge 1985b). The procedure involved 24-h postcapture observations of egg laying by females sorted immediately from the catch of a net tow (Runge 1985a, 1985b). The time of capture varied between 0600 and 2200. At all but one station, a portion of the catch, or the contents of a separate tow taken just before or after the live tow, was preserved in 4% buffered formaldehyde for later analysis.

Additional, preserved plankton samples were collected in June 1982 in an area centred on the western edge of German Bank during one of the above-mentioned cruises and in April 1983 along a transect through Browns Bank as part of the Southwest Nova Scotia Fisheries Ecology Program. In both cases, 60-cm paired Bongo nets fitted with flowmeters were towed obliquely from the surface to near bottom and back to the surface.

In the laboratory, between 40 and 60 *C. finmarchicus* females were sorted from each of the preserved samples. Each female was examined under a Wild stereomicroscope (12× or 25×) and the stage of maturity of oocytes in the oviducts was categorized and recorded. The criteria for classification (Table 1) were based on characteristics that could be quickly recognized at relatively low microscopic power. They are related but do not correspond exactly to the classification described for live *Calanus pacificus* (Runge 1985a).

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TABLE 1. Criteria for distinguishing stages of oogenesis of *Calanus finmarchicus*, based on characteristics of oocytes in oviducts of preserved females at 12× to 25× magnification. Note a change in terminology from table 2 in Runge (1985a): "anterior end of oviducts" has been substituted for "oviducal diverticula" to be consistent with terms in Blades-Eckelbarger and Youngbluth (1984).

Stage	Characteristics
1	No visible oocytes in anterior end of oviducts
3	Previtellogenic oocytes present in anterior end of oviducts. Ooplasm clear
4	Vitellogenic oocytes present in anterior end of oviducts. Oocytes >60 μm but <140 μm in diameter and contain dark brown ooplasm
5	Vitellogenic oocytes >140 μm present in anterior end of oviducts. Oocytes dark brown. Posterior end of oviducts still clear
7	Vitellogenic oocytes >140 μm in diameter present entire length of oviducts. Mature (postvitellogenic) oocytes appear as dark brown or dark gray bands, extending from the cephalic region to the last thoracic segment in lateral view

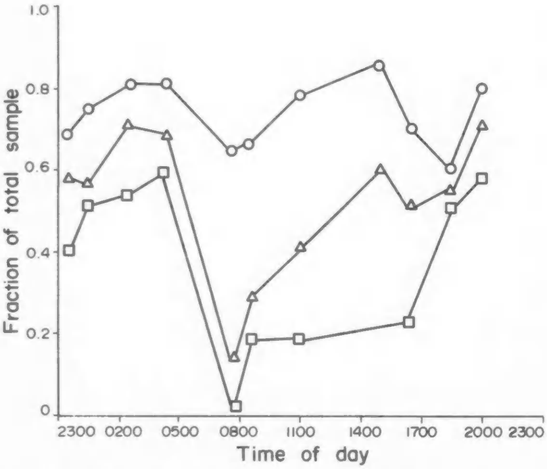

FIG. 1. Female gonadal development in preserved samples from German Bank transect. Abscissa is time of collection. Ordinate is proportion of sample in stage 7 (squares), stages 5–7 inclusive (ttriangles), or stages 4–7 inclusive (circles).

Results

It was first necessary to determine whether the stages of gonadal development described in Table 1 fluctuate on a daily cycle. A previous study showed that the proportion of stage 5–7 females in preserved samples of *C. pacificus* is a good predictor of spawning frequency (Runge 1985a). However, all the samples in that study were taken between sunset and midnight. Spawning in at least some *Calanus* populations occurs at night (Mullin 1968; Runge 1985a); the timing of egg laying in *C. finmarchicus* may be less synchronous (Marshall and Orr 1955), but a diurnal periodicity has sometimes been observed in this species as well (Harding et al. 1951). As is typical of cruises at sea, plankton tows were at various times of day and night in this study; in order to use all the data, an index that was not influenced by the time of sampling had to be found.

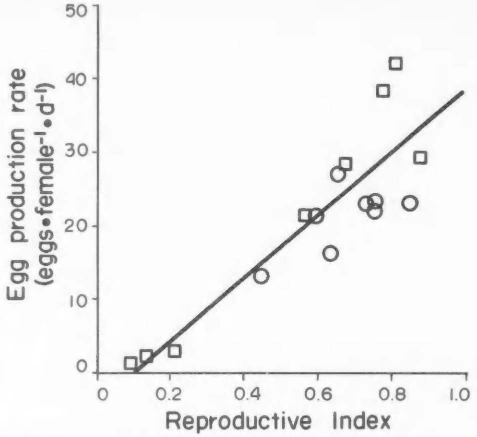

FIG. 2. Relationship between in situ egg production rate and reproductive index (proportion of females, stages 4–7 inclusive) from preserved samples collected concurrently in June 1982 (circles) and May 1983 (squares). Line is simple linear regression: $Y = 42.3X - 4.6$ ($r^2 = 0.75$).

I investigated diurnal variability in a series of preserved samples from the German Bank area, as they were taken at 1- to 4-h intervals over a 24-h period in an area of about 20 km diameter and it can be reasonably assumed that individual *Calanus* were experiencing similar environmental conditions (cf. Runge et al. 1985). The proportion of females carrying mature oocytes (stages 5–7) changed from a high of about 65% during the night to a low of about 10% in the early morning (Fig. 1). The pattern is consistent with Mullin's (1968) data suggesting that morning light stimulates egg release from female *Calanus helgolandicus* (*pacificus*). This indicates that an index based on stages 5–7 alone would only be appropriate for samples taken in the evening and perhaps later into the night. On the other hand, a cumulative index that includes females carrying early vitellogenic oocytes (stage 4) is much more constant. In this particular sampling period, the mean proportion of females with vitellogenic or postvitellogenic oocytes was 0.74 ($n = 11$) with a standard deviation of 0.084.

I determined a cumulative index based on stages 4–7 for samples of preserved females taken at the same time as egg production rate measurements (Fig. 2). A simple linear regression of egg production rate on this index has a slope significantly different from 0 ($P < 0.01$) and a correlation coefficient (r^2) of 0.75. If the abundance of *Calanus* females is known, this relationship can be used to determine daily production of *Calanus* eggs in the water:

$$E = (42.3(R) - 4.6)\ (A)$$

where E is the egg production rate (eggs per square metre per day), R is the reproductive index (relative proportion of stages 4–7), and A is the density of *Calanus* females (individuals per square metre).

Samples collected on 24–28 April 1983 were analyzed to illustrate the method. The reproductive index, and consequently the egg production rate per female, varied considerably from

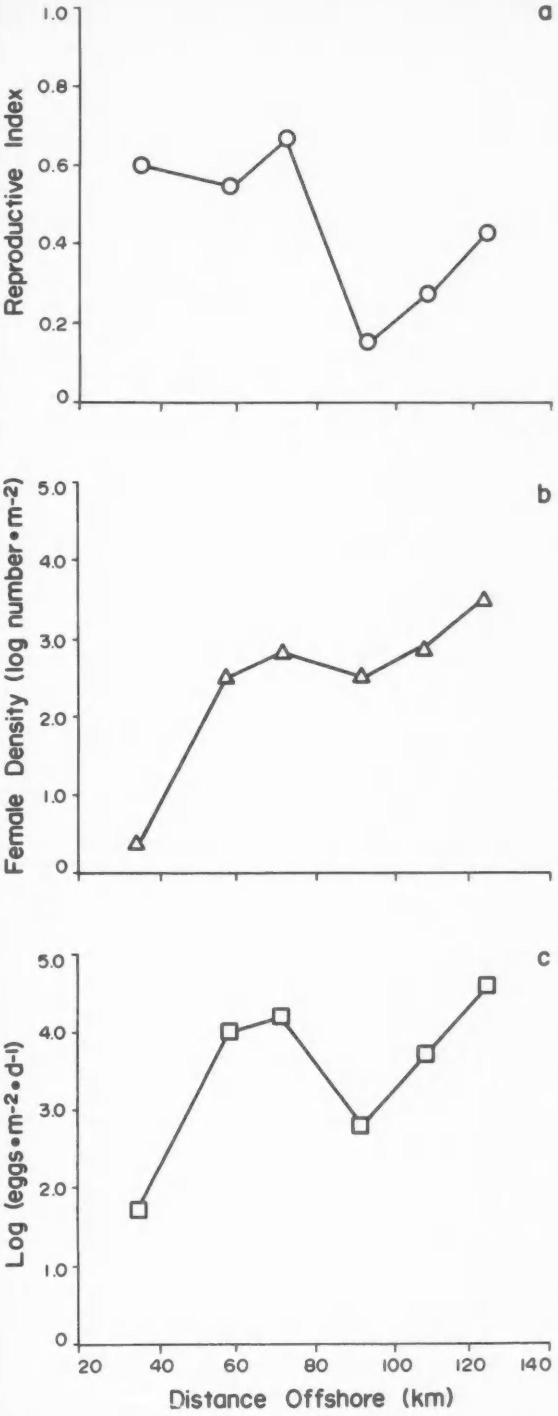

FIG. 3. (a) Reproductive index, (b) log of female abundance, and (c) of estimated egg production rate along Browns Bank transect, April 1983.

about 2 to 25 eggs·female $^{-1}$·d^{-1} (Fig. 3a). Female abundance varied by 3 orders of magnitude from about 2·m^{-2} at the shallow, inshore station to 3000·m^{-2} offshore (Fig. 3b). The combination of egg production rate and female abundance produced the spatial pattern of egg production rate (Fig. 3c). Egg production was about 50·m^{-2}·d^{-1} at the inshore station and about 40 × 10^{3}·m^{-2}·d^{-1} at the offshore station. Across Browns Bank, egg production varied by a factor of about 30, principally because of changes in fecundity, with the lowest values found directly over the Bank.

Discussion

There are several sources of error in the method that require further evaluation and testing. First, the criteria for assessing oocyte maturity may need refinement. While the characteristics of mature oocytes (stages 5–7) are quite obvious, identification of oocytes in earlier stages of vitellogenesis (stage 4) is more open to interpretation. Experiments are needed to determine error between observers and, if necessary, to make the evaluation process more quantitative. Duration of preservation does not appear to make a difference (reanalysis in 1987 of a sample from 1982 yielded the same reproductive index), but effects of different preservation techniques are unknown. Second, implicit assumptions in the use of a linear regression (Fig. 2) are that clutch size is constant and all females are capable of spawning. Relative to changes in the frequency of spawning, the clutch size variability is relatively small (Runge 1984); for similarly sized individuals of *C. finmarchicus* in waters off Nova Scotia, clutch size varied from about 40 to about 55 eggs·female^{-1}. Nevertheless, this source of error should be considered in analysis of residual variance. Finally, the robustness of the relationship in Fig. 2 needs to be tested. The data were collected in two different years but from the same general area: different areas and seasons, with diverse temperature and food regimes and possibly patterns of copepod behavior, may yield different relationships. Temperature affects egg production rate (Runge 1985b), for example, and the effect on oocyte maturation cycles is not known. Fortunately, in the Browns Bank area, water temperature rises relatively slowly; surface temperatures in April 1983 were 5–6°C (Dugas and Koslow 1984), similar to surface temperatures during the May 1983 cruise (K. Frank, Bedford Institute of Oceanography, Dartmouth, N.S., pers. comm.) and only 1–2° lower than temperatures found in late June 1982 (Runge et al. 1985). Later in the season and in other areas, however, water temperature may have to be taken into account.

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Predation on Larval Fish by Atlantic Mackerel, *Scomber scombrus*, with a Comparison of Predation by Zooplankton

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Pepin, P., S. Pearre Jr., and J. A. Koslow. 1987. Predation on larval fish by Atlantic mackerel, *Scomber scombrus*, with a comparison of predation by zooplankton. Can. J. Fish. Aquat. Sci. 44: 2012–2018.

This study reports on the feeding selectivity of Atlantic mackerel, *Scomber scombrus*, on larval fish (3–10 mm in length) presented as part of natural zooplankton assemblages. As with other vertebrate planktivores, prey preference was positively size selective, contrary to the pattern observed for several invertebrate carnivores. Larval fish density did not significantly influence predation rates. Furthermore, there was no evidence of either switching or saturation of the predator's functional feeding response with changes in alternative prey abundance, again unlike invertebrate predators. A review of previous experiments revealed that capture success of larval fish by predators is a function of the size of larval fish relative to the size of the predator, independent of predator taxa (i.e. vertebrate versus invertebrate).

Les auteurs ont étudié la sélectivité alimentaire du maquereau bleu (*Scomber scombrus*) pour des poissons larvaires (de 3 à 10 mm de longueur) faisant partie d'associations zooplanctoniques naturelles. Ils ont observé que la sélection des proies était positivement fonction de la taille, comme pour d'autres vertébrés planctonivores et contrairement à plusieurs invertébrés carnivores. Ils n'ont constaté aucune influence significative de la densité des larves de poisson sur les taux de prédation et ils n'ont observé ni changement ni saturation de la réponse fontionnelle de prédation lorsqu'était modifiée l'abondance d'autres proies secondaires, contrairement encore une fois aux prédateurs invertébrés. Une analyse des résultats d'expériences antérieures a révélé que le succès de capture des poissons larvaires par les prédateurs est fonction de la taille de ces poissons par rapport à la taille du prédateur, indépendamment des taxons des prédateurs (vertébrés ou invertébrés).

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Planktivorous fish have been hypothesized to be major if not dominant predators of young fish past the early larval period (Hunter 1981). This predator–prey interaction has been hypothesized to regulate recruitment to several fish stocks (Lett and Kohler 1976; Winters 1976; Skud 1982). However, the experimental or field data required to test these hypotheses are limited. The impact of adult fish on larvae cannot be assessed from stomach analysis because of rapid digestion of larval tissue (<30 min, Hunter and Kimbrell 1980; Pommeranz 1981), although this method yields good estimates of egg consumption (Harding et al. 1978; Hunter and Kimbrell 1980). Furthermore, most estimates of predation by pelagic fish

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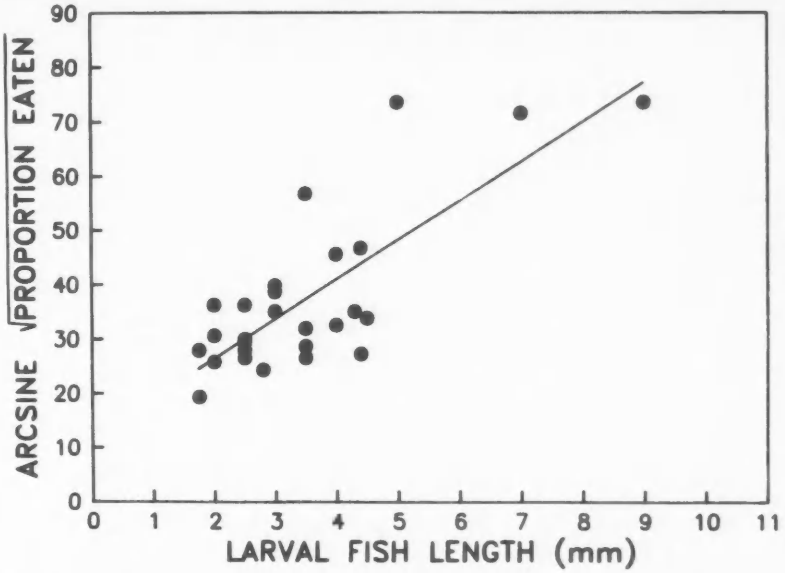

FIG. 1. Arcsine square root transformatin of the proportin of larval fish eaten by adult Atlantic mackerel as a function of larval fish length. The relationship was fitted by least-squares. $Y = 11.1 + 7.26X$, $r^2 = 0.61$.

upon fish larvae do not consider variability in prey selection and foraging behaviour, which are important determinants of pelagic fish foraging dynamics (Koslow 1981; Pepin et al. 1988).

We examine prey selection by adult Atlantic mackerel, *Scomber scombrus*, feeding upon natural zoo- and ichthyoplankton assemblages. In particular, we ask whether predation by Atlantic mackerel on larval fish varies in relation to larval fish size and alternative prey abundance. Zooplankton were used as alternative prey, since they constitute the principal prey of Atlantic mackerel (Sette 1950; Mackay 1979).

Materials and Methods

Atlantic mackerel were captured according to Lambert's (1982) specifications and kept in outside laboratory facilities (3000-L tanks) under natural light (approximately 14 h light: 10 h dark cycle) and temperature (16–18°C) conditions for 3–4 wk prior to experiments. All experiments were conducted between 1100 and 1400. Experiments were conducted only on days with cloud cover less than 30%.

Experimental procedures are described in detail in Pepin et al. (1988). Briefly, natural plankton assemblages (larval fish and zooplankton) used in each feeding trial were captured by towing a variety of ring nets (0.5–1.0 m in diameter, 80- to 150-μm mesh) at very low speed (<0.5 m/s) for 5 min on the morning of the experiment. Larvae so captured were generally in good physical condition, swam actively, and many survived for several days after capture. However, plankton nets may selectively capture weaker individuals within a population because of their lack of avoidance. Therefore, fish larvae captured by this method may be in poor condition relative to the natural population. This may result in an overestimate of the predation rate by Atlantic mackerel.

Experimental larval fish densities ranged from 10 to 150 larvae/m^3. Although these densities are high relative to natural conditions, they are required for statistical accuracy. The abundance of alternative prey ranged from 0.1 to 3.0 g dry weight/m^3. Although the upper limit of plankton concentrations used in the experiments may be high relative to mean plankton concentrations observed in the Gulf of St. Lawrence and Georges Bank (0.1–0.3 g dry weight/m^3, Lacroix and Filteau 1969; Bulleid and Stevens 1972; Stevens et al. 1973; Sherman et al. 1981), plankton concentrations within patches have been noted to reach concentrations 100–1000 times greater than the mean (Omori and Hamner 1982).

The predominant species of larvae in the assemblages were cunner (3–10 mm), *Tautogolabrus adspersus*, white hake (3–7 mm), *Urophysis tenuis*, and fourbeard rockling (3–7 mm), *Enchelyopus cimbrius*. These three species generally constituted 80% or more of the larval fish. Other species present included Atlantic mackerel (4–8 mm) and herring (7–10 mm), *Clupea harengus*. Alternative prey consisted almost entirely of copepods (*Pseudocalanus* sp., *Centropages typicus*, and *Calanus finmarchicus*). Prosome length ranged from 0.6 to 3.2 mm. The total abundance of alternative prey was measured by displacement volume and converted to grams dry weight (Weibe et al. 1975).

Each experiment was initiated by gently introducing a predetermined quantity of plankton into a tank with a school of 19 adult mackerel (mean length 35 cm, SD ±3 cm, total biomass 6 kg). The mackerel were allowed to feed for 5 min after the onset of the first feeding bouts, which generally occurred within 30 s of introducing the plankton. The fish were then herded into the adjacent tank using a small seine net (2.5 × 1 m, 10-mm mesh). The plankton was subsampled prior to the experiment, and the plankton remaining at the end was removed using a seine fitted with 0.333-mm mesh. Only one group of fish was used throughout the experiments. This removed a source of variability

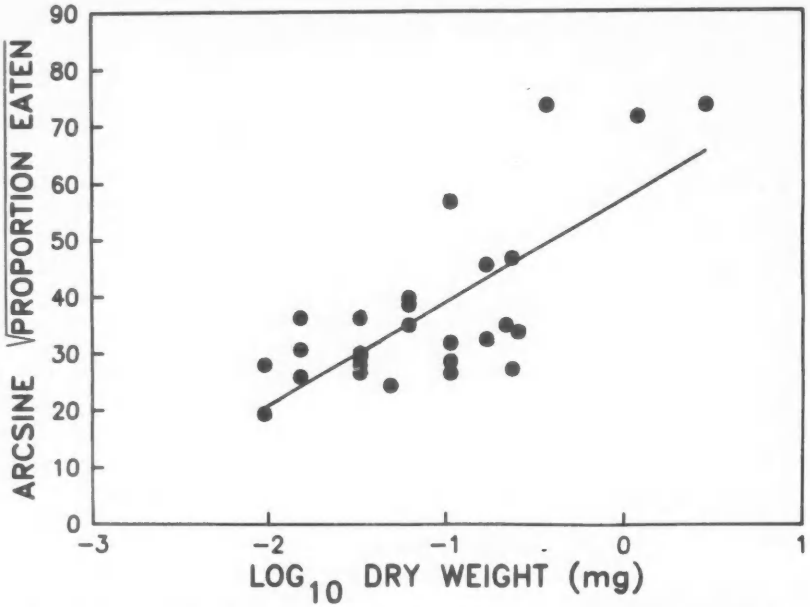

FIG. 2. Arcsine square root transformation of the proportion of larval fish eaten by adult Atlantic mackerel as a function of the logarithm of prey dry weight. $Y = 57.1 + 18.2X$, $r^2 = 0.54$.

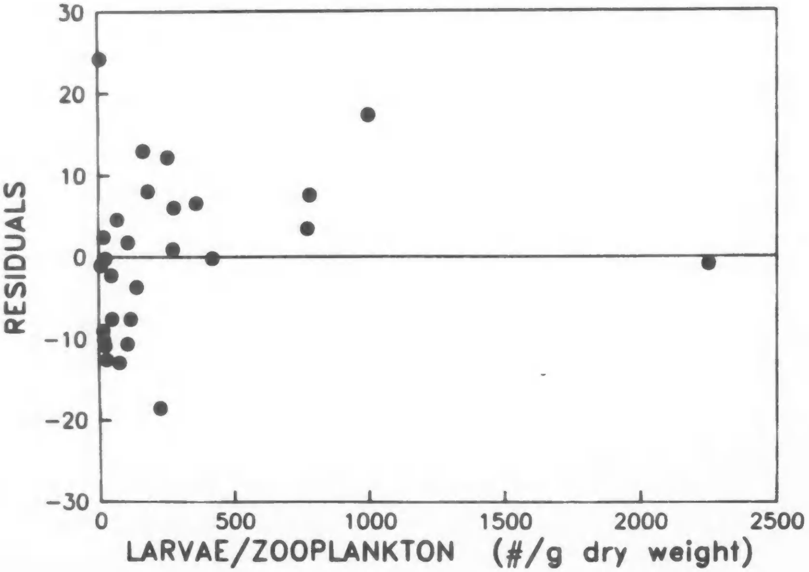

FIG. 3. Residuals from the regression of the arcsine square root transformation of the proportion of larval fish eaten as a function of larval fish length plotted against the density of larval fish relative to the density of zooplankton biomass.

and introduced the possibility of predator learning (Beukema 1968) which may limit our ability to generalize from the experiments.

Feeding selectivity was determined by comparing the size composition of fish larvae at the beginning and end of each feeding trial. After sorting, all larvae were placed into a 5-cm petri dish and photographed with Kodak Plus-X Pan (125 ASA) black and white film using a 35-mm SLR camera equipped with a 55-mm lens and a 5+ extension tube. Negatives were enlarged onto 20.3 × 25.4 cm photographic paper. Lengths were

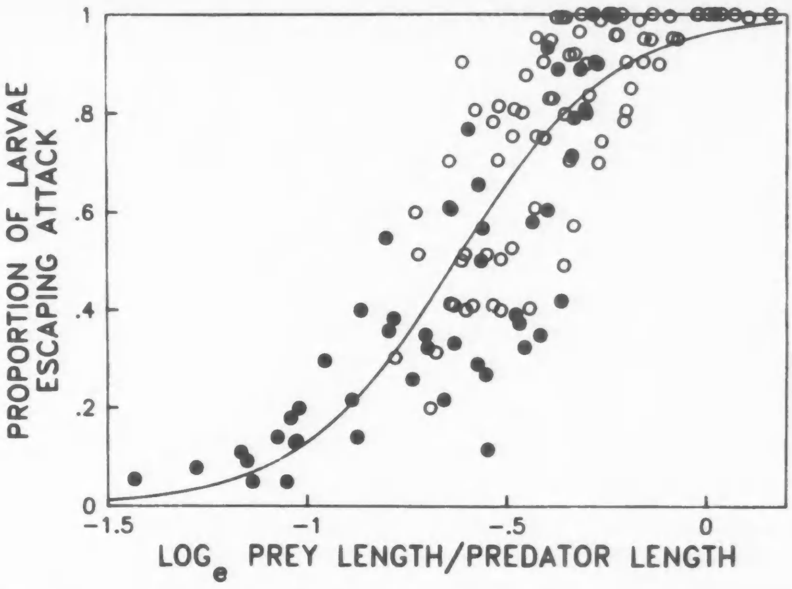

FIG. 4. Proportion of larval fish escaping attack as a function of the natural logarithm of the ratio of prey length relative to predator length. Solid circles represent data from experiments using vertebrate predators. Open circles represent data from experiments conducted using invertebrate predators. The curve fitted to the data represents a log-logistic fitted using maximum likelihood methods assuming binomial error structure; the equation is $Y = 1/\{1 + \exp[-b^*(X - m)]\}$ where $b = 5.095$ (SE = 1.02), $m = -0.628$ (SE = 0.049), Y is the production of larvae escaping attack, and X is the natural logarithm of the ratio of prey length relative to predator length.

measured with a Radio Shack TRS-80 digitizer. Larval fish lengths were converted to dry weights according to formulae calculated by Laurence (1978) and Fullerton (1983).

The feeding of mackerel on individual size classes (grouped by 0.5-mm length intervals) of larval fish was calculated as the proportion of larvae eaten ($[N_0 - N_t]/N_0$, where N_0 and N_t are the numbers of larval fish at the start and end of the feeding trial). To satisfy requirements of normality in parametric statistics, all proportions of larvae consumed were normalized using an arcsine square root transformation and are hereinafter referred to as % eaten.

Results

Stepwise multiple regression indicated that larval fish length was the only variable to significantly influence predation rates on larval fish by Atlantic mackerel ($Y = 11.1 + 7.26X$, where X and Y are the larval fish length (millimetres) and arcsine square root transformation of the proportion eaten respectively, $F = 41.1$, $P < 0.001$, $r^2 = 0.61$) (Fig. 1). After removing the effect of larval fish length on % eaten, neither larval fish density (partial correlation coefficient = 0.20, $P > 0.05$) nor total alternative prey abundance (partial correlation coefficient = −0.16, $P > 0.05$) had a significant influence on % eaten.

Similar results were obtained when predation rates were regressed against the logarithm of larval fish weight ($Y = 57.1 + 18.2X$, $r^2 = 0.54$, $P < 0.01$, where Y is the arcsine square root transformation of the proportion eaten and X is the logarithm (base 10) of the dry weight of fish larvae (milligrams) (Fig. 2). However, we shall confine further results and discussion to the relationship between predation rates and larval fish length, unless indicated otherwise.

Larval fish assemblages were generally dominated either by cunner larvae or by a combination of hake and rockling larvae, which are very similar at lengths less than 10 mm (Fullerton 1983). If dominance by either group had influenced % eaten, residuals from the regression of % eaten versus larval fish length would tend to be either positive or negative. However, there was no apparent species specific difference in the residuals (Mann–Whitney U test, $n_1 = 14$, $n_2 = 14$, $U = 77$, $P > 0.05$). Bailey (1984) noted a similar pattern when comparing predation rates of *Aurelia aurita* on five species of larval fish (cod, herring, flounder, plaice, turbot).

To analyze the data for evidence of switching (Murdoch 1969), we examined whether predation on larval fish varied as a function of the relative proportion of larval fish in the experiment. Total zooplankton biomass determines the feeding response of Atlantic mackerel (Pepin et al. 1988), so larval density was normalized to zooplankton biomass (number per gram dry weight). No significant switching appeared to take place, as seen from a plot of residual predation rate (i.e. residuals from the regression of % eaten as a function of larval fish length) and normalized larval fish density (Fig. 3) ($r = 0.22$, $P > 0.05$, $n = 28$). However, residual predation rates tend to be positive for normalized larval fish densities greater than 400 larvae/g dry weight of zooplankton. Although this is not significant (binomial test, $P(r,t,0.5) = 0.09$, $r = 5$, $t = 6$), adult Atlantic mackerel in the laboratory may begin to feed more

TABLE 1. Characteristics and sources of data used in the analysis of capture success as a function of predator and prey size.

Prey species	Length of larval fish (mm)	Predator species and length	Source
Northern anchovy, *Engraulis mordax*	2–4	Juvenile *Euphausia* (8 mm)	Theilacker and Lasker 1974
	3.2–10	*Amphiprion percula* (44 mm)	Webb 1981
	6–34	*Engraulis mordax* (85 mm)	Folkvord and Hunter 1986
	7–51	*Scomber japonicus*	Folkvord and Hunter 1986
Cape anchovy, *Engraulis capensis*	4–17.5	*Engraulis capensis* (15–30 mm)	Brownell 1985
Atlantic herring, *Clupea harengus*	9.8–18.4	*Aurelia aurita* (12–45 mm)	Bailey and Batty 1984
Atlantic cod, *Gadus morhua*	4.2–5.9	*Aurelia aurita* (12–22 mm)	Bailey and Batty 1984
Plaice, *Pleuronectes platessa*	6.6–8.2	*Aurelia aurita* (12–22 mm)	Bailey and Batty 1984
Flounder, *Platichthys flesus*	3.4–3.7	*Aurelia aurita* (12–21 mm)	Bailey and Batty 1984

heavily on larval fish as larval fish increase in density relative to zooplankton. However, such high relative densities of larval fish are seldom encountered in the field.

Discussion

The pattern of prey removal observed in our study of Atlantic mackerel is similar to that observed by Folkvord and Hunter (1986) for chub mackerel, *Scomber japonicus*. Although predation rates were somewhat higher in our experiments, 100% of 7-mm larvae consumed in 5 min versus 100% of 16-mm larvae in Folkvord and Hunter's (1986) experiments, the mean length of mackerel was almost twice as great (35 cm versus 19 cm), and the number of fish per cubic metre was three times higher in our study than in Folkvord and Hunter's. The overall similarity in the pattern of positive prey selection for both *Scomber scombrus* and *Scomber japonicus* suggests that the components of their feeding behaviour for larval fish may also be similar.

Positive prey selection by vertebrate predators has been noted in several studies (see Greene 1985 for review). Prey preference by vertebrate planktivores for fish larvae appears to be the inverse of that observed for invertebrate carnivores (e.g. euphausiids, chaetognaths, and medusae, Theilacker and Lasker 1974; Kuhlmann 1977; Bailey and Batty 1984). Adult mackerel prey more heavily on large larvae than on smaller ones, while studies of predation by various invertebrates show that they are generally best able to capture larval fish at the stage of yolk absorption, after which their capture success declines rapidly as the larvae grow (Lillelund and Lasker 1971; Bailey and Yen 1983; Bailey 1984). Although encounter rates between larvae and invertebrate predators increase as larval fish grow (Gerritsen and Strickler 1977; Bailey and Batty 1983), higher burst swimming speeds facilitate escape by larger larval fish (Webb 1981; Bailey 1984). Larvae less than 10 mm total length could hardly escape by swimming from adult mackerel. Average adult mackerel swimming speed recorded by Pepin et al. (1988) and Mackay (1979) ranged from 30 to 60 cm/s (approximately 0.9–2.1 body lengths/s), while measurements of larval fish burst swimming speed range from 3 to 10 body lengths/s (approximately 1–10 cm/s) (Bishai 1959; Houde 1969; Webb and Corolla 1981; Webb 1981).

Although vertebrates and invertebrates have been hypothesized to have distinct patterns of predation (Holling 1965), their apparent dichotomy in selection for larval fish may result primarily from simple differences in size of most vertebrate and invertebrate predators. Predators generally exhibit a Gaussian distribution of preferred prey size (Ivlev 1961). Prey less than the most highly selected size range presumably confer insufficient energetic return, while those larger than the optimal size range are presumably able to escape the predator (Bailey and Batty 1984; Brownell 1985; Folkvord and Hunter 1986). Although the escape response is not the only component of the predation process, it does provide insight into the maximum prey size that a preditor can handle as well as into the response of the prey to attack.

To determine whether capture success of larval fish was influenced by predator type, we compared capture success of vertebrate and invertebrate predators as a function of their size relative to the size of larval fish prey (for data sources, see Table 1). Using maximum likelihood methods, we fitted a log-logistic curve to the proportion of larval fish escaping attack during feeding experiments as a function of the natural logarithm of the ratio of larval fish length/predator length (Fig. 4). A Mann–Whitney U-test revealed no significant difference between residuals from experiments conducted using vertebrate predators relative to those using invertebrate predators ($U = 2268$, $N_1 = 49$, $N_2 = 80$, $0.10 < P < 0.05$). These results suggest that the apparent dichotomy of positive size selection for vertebrate predators and negative size selection for invertebrate predators for larval fish less than 10 mm results largely from the influence of relative predator size on capture success, i.e. that vertebrate predators are generally larger and therefore select larger prey. One hundred percent escapement appears to occur when the length of larval fish is 67% of the length of a given predator. Absolute predation rates will, however, also depend on encounter rates and the reaction field of the predator.

Adult mackerel show positive size selection for both larval fish (this study) and copepods (Pepin et al. 1988). However, predation rates on copepods of a given weight are generally 20–30% higher than predation rates on fish larvae of the same weight. This result is in contrast with Koslow's (1981) suggestion that prey selection by northen anchovy was a function of prey weight independent of taxa. However, he did not observe predation upon larval fish. The apparently different susceptibility of larval fish to a visual predator may be due to differences in shape, pigmentation, or avoidance ability.

The abundance of alternative prey had no significant influence on the proportion of larval fish eaten, either through switching or through saturation of the predator's short-term functional response. This is in contrast with the response of several invertebrate carnivores which show a strong preference for copepods relative to fish larvae (Kuhlmann 1977; Bailey and Yen 1983; Purcell 1985) and in contrast with most other studies on predation by fish (Beukema 1968; Forney 1974; Holčík 1977; Gibson 1980; Nielsen 1980; Brownell 1985). This may be due to differences in the size of the different prey types used in previous experiments. However, although alternative prey abundance does not appear to influence the short-term feeding behaviour of Atlantic mackerel, variations in alternative prey abundance may influence the time required by fish predators to satiate (Nielson 1980; Brownell 1985; Pepin 1987). Therefore, before we can conclude that alternative prey abundance does not influence predation by mackerel on larval fish, the effect of variations in total food abundance on the feeding and growth of planktivorous fish in the wild must be assessed.

The influence of larval fish condition on predation rates by adult mackerel, which is considered an important determinant of vulnerability to predation (Bams 1967; Webb and Corolla 1981), was not investigated in our study. However, larval fish condition is most variable at the time of yolk absorption because of the need for immediate nourishment and the limited feeding efficiency of larval fish (Beyer and Laurence 1980). Because foraging velocity of adult mackerel is so much greater than the escape velocity of larval fish, changes in larval fish condition may have an insignificant influence on the ability of large active planktivores to capture larval fish. However, since condition and larval fish growth are linked, the influence of the larval fish feeding environment on the cumulative impact of pelagic fish predation could take place on a time scale longer than the duration of most experiments.

Our study indicates that larval fish may be vulnerable to predation by pelagic fish for longer periods of time than to predation by invertebrate carnivores due to differences in their relative size. However, details of encounter rates and predator biomass are required to evaluate the potential influence of the different predator types on larval fish survival. As well, the potential interaction between larval fish, their predators, and other trophic levels (e.g. zooplankton which serve as prey for both fish larvae and their predators), which may significantly influence larval fish survival (Shepherd and Cushing 1981; Pepin 1987), should be investigated.

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A Time-Saving Filtration System For Nutrient Analysis

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Bowman, G. T., B. A. Schuknecht, J. E. Wilken, and W. C. Sonzogni. 1987. A time-saving filtration system for nutrient analysis. Can. J. Fish. Aquat. Sci. 44: 2018–2021.

Conventional membrane filtration to separate particulate material from water samples, such as in the analysis of dissolved nitrogen and phosphorus, is time consuming and subject to contamination. To minimize this problem, a device is presented that allows filtration of up to 80 samples per hour without introducing contamination or affecting the sample integrity. The device requires only partial dismantling and minimal cleaning with dilute hydrochloric acid between samples. Validation tests show that the device does not alter dissolved constituents in filtered samples. Unlike conventional membrane filtering apparatus, a cumbersome vacuum flask is not required. The system is compact and easily adapted to mobile and shipboard laboratories.

La technique classique de filtration sur membrane utilisée pour isoler les particules d'échantillons d'eau, notamment au cours de l'analyse de l'azote et du phosphore dissous, est très longue et sujette à contamination. Dans le but de contrer ce problème, les auteurs présentent un dispositif permettant de filtrer jusqu'à 80 échantillons à l'heure sans contaminer ou modifier l'intégrité de ces derniers. Le dispositif ne nécessite qu'un démontage partiel et un minimum de nettoyage à l'acide chlorhydrique dilué entre les échantillons. Des tests de validation ont montré que cette méthode ne modifiait pas les constituants dissous des échantillons filtrés. Au contraire de l'appareillage classique de filtration sur membrane, il n'est pas nécessaire d'utiliser un erlenmeyer à vide encombrant. Le dispositif est compact et facilement adaptable aux laboratoires mobiles ou installés à bord de bateaux.

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Membrane filtration is a common technique used to separate particulate constituents from water, but can be time consuming, labor intensive, and a source of troublesome contamination. Problems with filtration are particularly apparent when processing large numbers of samples, such as in the analysis of many lake or seawater samples for nitrogen (N) and phosphorus (P). Centrifugation is an alternative to filtration, but is not an accepted method as it does not always provide complete removal of particulates (American Public Health Association 1980).

Filtration procedures include gravity filtration and vacuum filtration. Conventional vacuum filtering techniques, such as

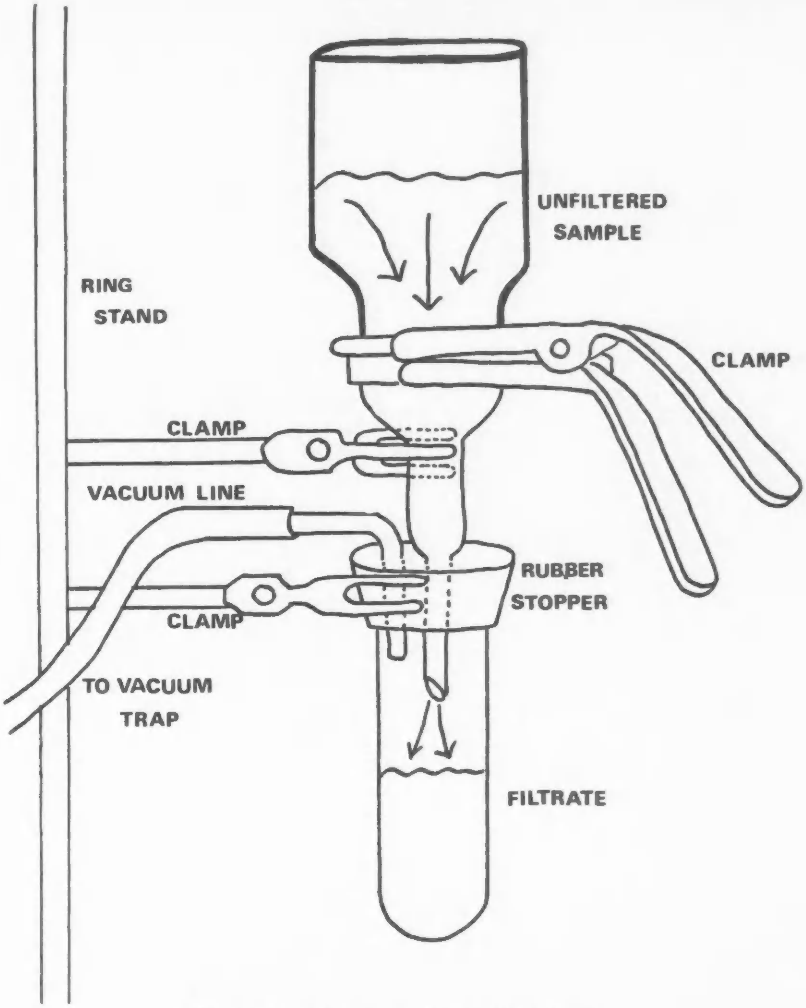

FIG. 1. Schematic representation of filter apparatus.

described by Strickland and Parsons (1972), are much quicker than gravity filtering procedures and are used almost exclusively in environmental laboratories. However, when measuring some analytes, such as low levels of dissolved P and N, vacuum systems must also be completely dismantled and cleaned between every sample in order to avoid contamination. For example, Eaton and Grant (1979) observed that glass frit filter supports commonly used in membrane filtering apparatus can adsorb ammonia, leading to significant errors in nitrogen determination.

Several modifications of commercially available systems have been suggested to improve the filtration process (Nelson 1983; Randall 1984), but they require both the flask and filtration assembly to be disassembled, cleaned, and reassembled between filtrations. We present here a simple setup that allows rapid, contamination-free filtration, that minimizes loss of analyte through adsorption onto the apparatus, and that requires only minimal dismantling between samples. The system is now used routinely in our laboratory, where about 20 000 samples are filtered annually for N and P analyses alone. The system permits the filtering of nearly three times as many samples as can be done using conventional apparatus. On the average, about 65 samples per hour can be filtered, but up to 80 samples per hour can be filtered under optimum conditions. The number of samples that can be filtered per hour is, of course, influenced by the characteristics of the sample and the skill and experience of the technician.

Apparatus

Our device, shown in Fig. 1, is a modification of a commercially available (Millipore Corporation, Bedford, MA) glass membrane filtering apparatus. The funnel and base are teflon-faced pyrex glass with a coarse frit glass filter support. The modification, requiring the services of a glass blower, entails replacing the stem with a 60-mm-length by a 6-mm-diameter piece of Pyrex tubing. The base is mounted in a No. 11 neoprene stopper in one of two 4-mm-diameter holes. A 90°

TABLE 1. Evaluation of ammonia, nitrate plus nitrite, and P carryover contamination in deionized–distilled water blanks filtered after an actual sample. Concentrations in mg $N \cdot L^{-1}$ or mg $P \cdot L^{-1}$.

Domestic wastewater sample	Ammonia		Nitrate plus nitrite		Dissolved reactive P	
	Sample	Carryover	Sample	Carryover	Sample	Carryover
Raw 1	12	<0.02	0.6	<0.02	1.55	<0.002
Treated 1	0.3	<0.02	13.2	<0.02	1.76	<0.002
Raw 2	10	<0.02	0.1	<0.02	0.06	<0.002
Raw 3	21	<0.02	—	—	1.22	<0.002
Treated 3	15	<0.02	0.9	<0.02	0.14	<0.002
Raw 4	26	<0.02	—	—	1.03	<0.002
Treated 4	6.1	<0.02	13.8	<0.02	0.32	<0.002
Treated 5	14	<0.02	1.1	<0.02	2.5	<0.002
Raw 6	11	<0.02	0.1	<0.02	3.0	<0.002
Treated 6	5.3	<0.02	2.4	<0.02	0.32	<0.002

glass elbow is placed in the second hole and attached to a vacuum source. The rubber stopper is attached to a heavy duty ring stand with a three-fingered clamp. The apparatus occupies about 1 linear ft of bench space. A total of 3 linear ft of space is required for the apparatus and supplies necessary to perform filtrations. Filtration is accomplished by placing a culture tube over both openings, thereby creating a vacuum within the tube and subsequently causing the sample to be pulled through the filter into the tube (Fig. 1). Note that although the funnel and base of our device are entirely glass, the device may be fabricated from other materials such as polyethylene or stainless steel depending on the particular application.

An optional clear safety shield, fabricated from a 20.3-cm-diameter (8 in.) Plexiglas cylinder, can be easily attached to the ring stand with a clamp. The shield protects the technician from being splashed in the event of an accident. The shield is clear, extends about 12 cm from the funnel, and provides adequate room for the technician to maneuver.

Filtering Procedure

Once a filter is placed in the apparatus and the funnel secured with the aluminum clamp, the sides of the funnel and filter are rinsed with about 10–15 mL of hydrochloric acid (3.6 *N* acid is usually used). The stem of the apparatus is also rinsed with acid to prevent contamination from the previous sample. A 250-mL polyethylene Erlenmeyer flask is placed under the neoprene stopper and the acid solution pulled through the apparatus. With the flask in place, the apparatus is rinsed with three separate 25-mL portions of distilled–deionized (D-DI) water. When the last remaining water has been evacuated from the apparatus, the flask is removed, the stem rinsed with a few millilitres of D-DI water, and the solution discarded. The sample is then poured into the funnel; about the first 10 mL is filtered into the flask and discarded. The remaining sample is then filtered into a clean 20 × 150 mm disposable borosilicate glass culture tube and covered with laboratory film (Parafilm, American Can Company, Greenwich, CT). Alternatively, the sample may be filtered into a 60-mL polyethylene bottle and capped. The culture tube or polyethylene bottle is removed from the apparatus when the container is about three quarters full to prevent the sample from coming in contact with the rubber stopper. If the sample comes in contact with the rubber stopper, the apparatus is tipped to the side slightly and the underside of the stopper is rinsed with about 10 mL of 3.6 *N* HCl followed by 25 mL of D-DI water. Next, the funnel is removed and rinsed with about 10 mL of 3.6 *N* HCl followed by about 25 mL of D-DI water. The filter is then discarded and the filtering process repeated. Blanks consisting of D-DI water are filtered at the beginning and end of each batch of filtrations to monitor cleaning efficiency and potential carryover contamination. About 10% of the samples are filtered in duplicate. The duplicated filtrations serve to monitor precision and the integrity of the filtration.

Experimental

Carryover contamination was evaluated by filtering 12 domestic wastewater (both raw and treated) samples containing ammonia and nitrate ranging from 1 to 26 mg $N \cdot L^{-1}$ and dissolved reactive P ranging from 0.14 to 3.0 mg $P \cdot L^{-1}$. D-DI water blanks were filtered after each of the wastewater samples to assess carryover. The filtrate was analyzed for ammonia N, nitrate plus nitrite N, and dissolved reactive P. The ammonia and nitrate plus nitrite were analyzed simultaneously using the automated phenate and cadmium reduction techniques, respectively (U.S. Environmental Protection Agency 1979). The dissolved reactive P analyses were determined using an automated ascorbic acid technique (U.S. Environmental Protection Agency 1979). Results are shown in Table 1. Note that the detection limit for ammonia N, nitrate plus nitrate N, and dissolved reactive P was 0.02, 0.02, and 0.002 $mg \cdot L^{-1}$, respectively.

In addition to contamination, the possibility that analytes may react with or adsorb onto the walls of the filtering apparatus was also evaluated. Adsorption of P is of particular concern, since orthophosphate has a strong tendency towards adsorption. For example, it is known that P may adsorb to glass containers (Ryden et al. 1972).

To assess the potential loss through adsorption, replicate aliquots of several dissolved reactive P standard solutions were filtered in the same manner as typical samples. Additional replicate aliquots were retained unfiltered for reference. Both filtered and unfiltered standards were analyzed for dissolved reactive P using the automated ascorbic acid technique (U.S. Environmental Protection Agency 1979). Results are presented in Table 2.

TABLE 2. Evaluation of P adsorption on the filtering device based on standard solutions analyzed after filtration.

	Dissolved reactive P ($mg P \cdot L^{-1}$)	
Number analyzed	Filtered (mean)	Unfiltered[a] (mean)
3	0.007	0.006
3	0.006	0.006
2	0.006	0.006
2	0.051	0.051
3	0.051	0.050
2	0.006	0.006

[a] Unfiltered standards analyzed for reference.

Discussion

The results from the carryover evaluation (Table 1) show no apparent carryover contamination for N or P in the D-DI blanks filtered immediately after the wastewater samples. The blanks were all less than the detection limits of the analytical techniques used. Additionally, our experience in running several thousand blanks in association with routine analyses indicates that the HCl rinsing procedure adequately removes traces of N and P from the apparatus.

Occasional problems have been observed when filtering oily wastes, such as dairy plant wastewater. These wastes cause a coating or film to appear on the funnel and stem. The problem can be avoided or corrected by periodically cleaning the apparatus with NOCHROMIX (Godax Laboratories), a nonmetallic sulfuric acid cleaning solution.

Cleaning is facilitated by assembling the apparatus without the filter, pouring about 150–200 mL of Nochromix into the funnel and drawing the solution into an Erlenmeyer flask. The acid residue is then flushed from the apparatus with cold tap water followed by copious volumes of D-Di water. Cleaning frequency depends on the type and number of water and wastewater samples. Best results are obtained through regularly scheduled cleaning.

The filtering device is used for the preparation of almost all samples for N and P analyses at the Wisconsin State Laboratory of Hygiene. The adsorption assessment is based primarily on dissolved reactive P, since phosphate is the one constituent most apt to adsorb or react with the device itself. The data in Table 2 show that filtered standards did not differ significantly from the unfiltered standards ($p = 0.05$ using Student's *t*-test). The data indicated that P adsorbtion does not occur at a measurable level using the technique currently employed by the laboratory. We did not observe any P loss, probably because of the relatively short contact time within the filtering device. Ammonia adsorption was not specifically evaluated in this study, since past work in our laboratory and elsewhere (Eaton and Grant 1979) indicates that any amount of ammonia likely to be adsorbed would be small relative to the levels of environmental concern. For all practical purposes, constituents do not adsorb to the apparatus in our particular application. However, if the apparatus is used for other applications, such as preparing samples for dissolved metal analyses, further evaluation is recommended.

Conclusions

Prior to the development of the filtration apparatus and technique described herein, our laboratory relied on gravity filtration. However, because of space restrictions and changes in environmental regulations, gravity filtration was abandoned for a vacuum technique. Existing vacuum techniques allowed about 30 samples per hour to be processed. However, by making some simple modifications to a standard glass membrane filtering apparatus, about 80 samples per hour can be processed. The design also facilitates cleaning to minimize contamination among samples. Because of its small size and ease of use, the apparatus could be easily adapted to mobile and shipboard laboratories.

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A Synoptic View of the Gulf Stream Front with 70-kHz Sonar: Taking Advantage of a Closer Look

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Acoustical scattering across the near-surface frontal zone of the Gulf Stream off Cape Hatteras was greatest in the thermal front. Little biological scattering was evident in the colder Slope water, but in the Gulf Stream, scatterers formed five horizontal bands. Interpretation and new applications of acoustical information in biological oceanography are discussed.

La diffusion acoustique dans la zone frontale près de la surface du Gulf Stream au large de Cape Hatteras est plus élevée dans le front thermique. On a observé peu de diffusion biologique dans les eaux de versant plus froides mais, dans le Gulf Stream, il y avait cinq bandes horizontales. On discute de l'interprétation et des nouvelles applications des données acoustiques en océanographie biologique.

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We present a striking example of 70-kHz acoustical scattering across the northeast edge of the Gulf Stream in relation to temperature, vertical change in temperature, and depth from 14 to 150 m (Fig. 1). Because sound scattered by density gradients is negligible, we attribute the scattering to the biota present. Organisms that scatter this frequency of sound are generally 1 cm or more long and include some of the largest zooplankton and all of the nekton, animals large enough to swim independently of water movements.

Nekton aggregate in frontal zones (Bowman and Esaias 1978; Owen 1981). Sonar techniques make possible the study of fine-scale biological structure in dynamic frontal zones. Murav'yev and Shirshov (1984), for example, have found that nektonic organisms are concentrated spatially and temporally at water density gradients. Our sonar transect displayed in Fig. 1 highlights the pattern in the distribution of small nekton at an ocean front including high concentrations in the areas of maximum change in physical parameters.

The traditional hypothesis proposed to explain the concentration of biota in a frontal zone is that primary and secondary producers are passively concentrated by the physical dynamics to locations where nutrients and light stimulate production. An alternative hypothesis, which may be consistent with the first, has been proposed from the perspective of those who study animals in which behavior is not a passive, but rather an active reponse to the environment. This hypothesis suggests that the concentration of biota in certain areas results from the direct behavioral responses of fish or other nekton to environmental factors such as temperature and food abundance (Crowder and Magnuson 1983). Answering these questions and other biological questions in complex dynamic areas like the Gulf Stream front would perhaps be impossible without an ability to obtain rapid synoptic views of biota such as pictured here.

Methods

The acoustical scattering and temperature profile were obtained from the R/V *Cape Hatteras* on 12 July 1983, along a 14.5-km transect 115 km east–northeast of Cape Hatteras from 35°47.98′N, 74°37.67′W (1248) to 35°41.25′N, 74°28.80′W (1545). The northeast to southwest direction of the transect progressed from Gulf Stream water overlying Continental Slope water to Gulf Stream water alone. A Simrad EY-M echosounder with an 11° beam width was operated at 70 kHz with a pulse duration of 0.6 ms. The transducer faced downward and was towed 4 m below the surface. An echo processor (Powell and Stanton 1983) simultaneously calculated echo peaks and the integrated echo in individual 1-m depth intervals and transferred the processed data to an Apple II microcomputer which, in turn accumulated echo data from 120 transmissions (approximately 2 min) to obtain the (average) integrated echo and a probability density function (PDF) of echo peaks. Data were analyzed from 12 to 150 m, owing to the range limitations of this sonar system. The arithmetic mean of the relative integrated echo was calculated for each water type and was converted into the commonly used volume scattering strength which is on a decibel scale (Urick 1975; Clay and Medwin 1977). We determined the resolution of scatterers from an examination of PDF values (Stanton 1985a, 1985b).

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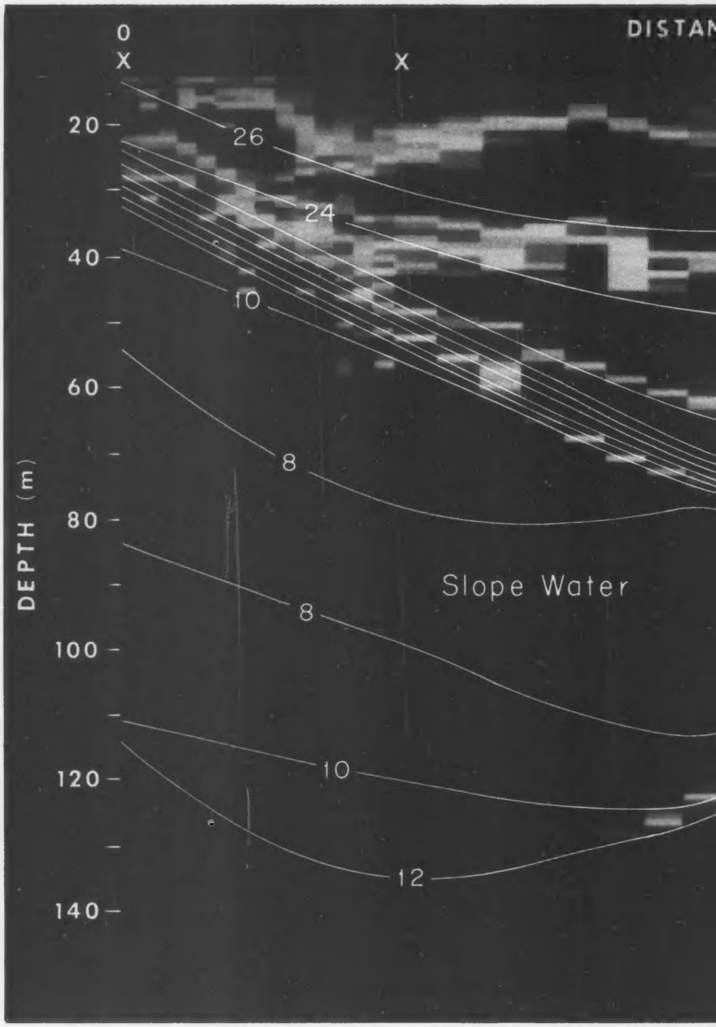
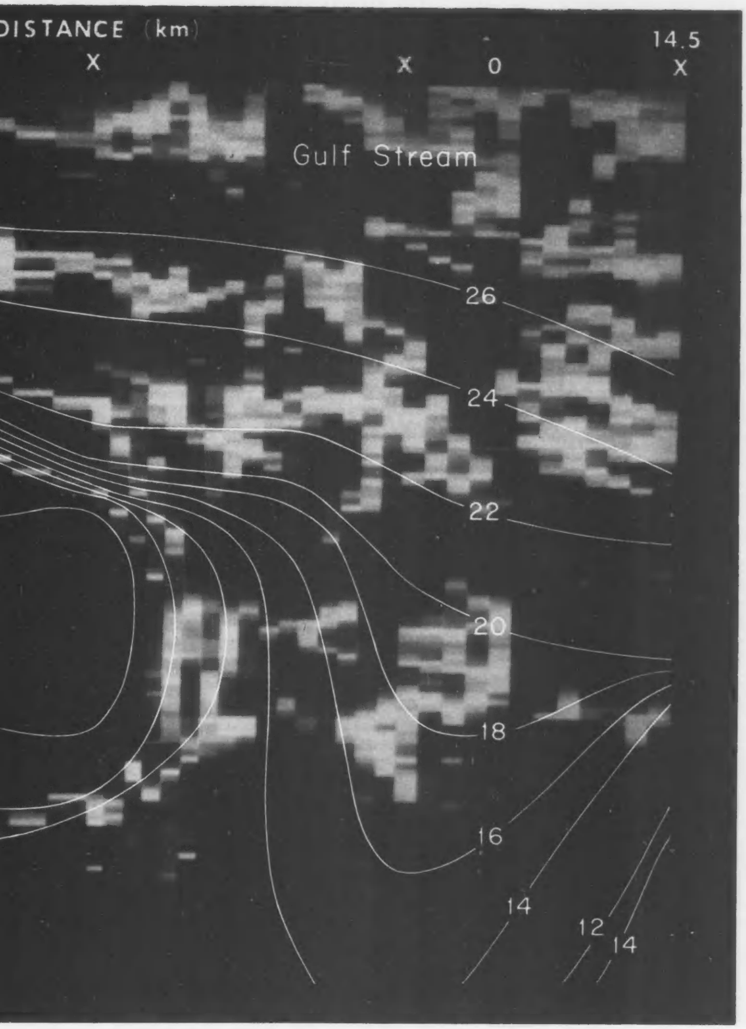


FIG. 1. Relative abundance of biological scatterers of 70-kHz sound in relation to dep
zone of the Gulf Stream during the daytime on July 12, 1983, 115 km east-nort
approximately 300 m, and the color scale is logarithmic in 21 steps passing from bl
×. 0 indicates the location of a 2-min column of acoustic data deleted owing to int



tion to depth and temperature along a 14.5-km transect crossing the near-surface frontal
east-northeast of Cape Hatteras. Vertical resolution is 1 m, horizontal resolution is
ng from blues to greens to yellows to reds. Locations of the XBT casts are indicated by
ving to interference from porpoise sounds. See methods for more explanation.

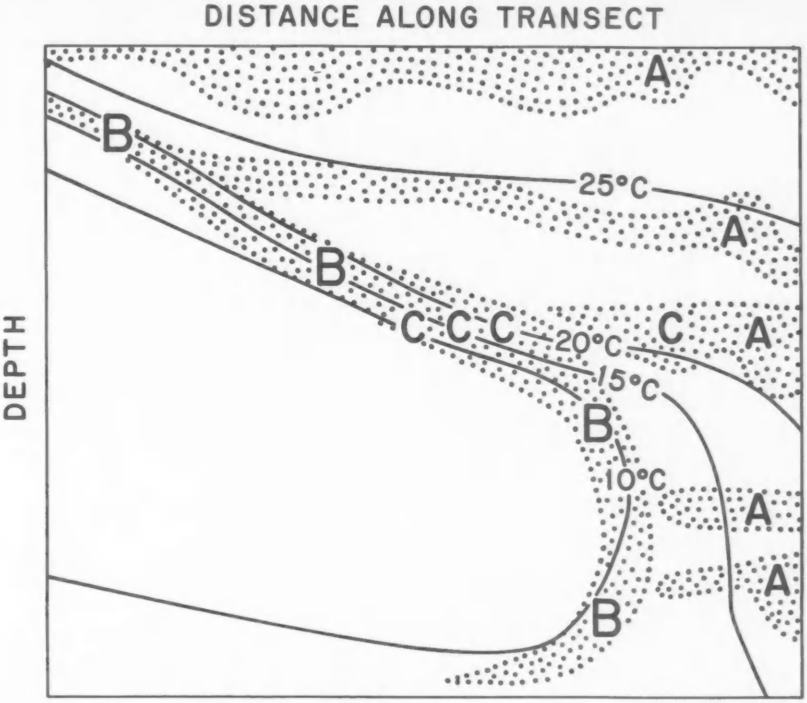


FIG. 2. Location of net samples to test whether (A) each acoustic surface is biologically distinct, (B) the biological structure of the same acoustic surface changes with depth, and (C) the biological structure of an acoustic surface changes with temperature. Letters on the figure show locations for replicated net sampling. Stippled areas are acoustic surfaces approximated from Fig. 1.

We contoured the temperature profile from five expendable bathythermograph (XBT) releases along the transect. Changes in temperature with depth (degrees Celsuis per metre) also were calculated, smoothed over 3 m, and contoured.

Results

Throughout the area, echoes of the individual scatterers overlapped each other (i.e. the organisms were not resolvable by sonar), indicating minimum densities of organisms (>1 cm long) of at least $28/1000\ m^3$ at 20 m to at least $0.5/1000\ m^3$ at 150 m.

The transect was divided into three water types, Slope water (<12°C), Gulf Stream water (>20°C), and thermal boundary water between the Slope and Gulf Stream waters (Fig. 1). The three types of water differed greatly in biological sound scattering characteristics. Slope water, the uniform blue area in the lower left corner of Fig. 1, had the lowest scattering strength (mean −83 dB where the decibel level represents the fraction of acoustical energy returned from one cubic metre of water on a logarithmic scale. For example, if the fraction is $10^{-8.3}$, the "dB" level is −83). Scattering strength in the thermal boundary water, the red diagonal, J-shaped band (Fig. 1), was extremely heterogeneous. In Gulf Stream water, the area in the upper and right side (Fig. 1), scattering strength revealed reddish yellow horizontal striations in the distribution of organisms.

In the thermal boundary water the integrated echo data indicated relatively high concentrations of scatterers in a continuous band located at approximately 20 m at the northwestern end of the transect and sloping to approximately 80 m near the middle of the transect. Scattering strength varied from −76 dB between 28 and 55 m to −68 dB in the most intense scattering area between 51 and 77 m and −74 dB between 72 and 95 m. In the intense scattering area at 60–70 m (Fig. 1) the echo signal was so strong that it saturated the processor amplifier; thus the values underestimate relative integrated echo strength. In the deeper portion of the thermal boundary, scattering follows the 10–12°C isotherms and recurves into the Slope water.

In the Gulf Stream the distribution of scatterers was horizontally striated but the scattering strength averaged −75 dB. Four (possibly five) horizontal striations are evident, located at approximate depths of (I) <12 m, (II) 35–50 m, (III) 60 m, (IV) 90–100 m, and (V) 100–110 m. The three deeper striations (III, IV, and V) appear to cross into the sharp thermal gradient whereas striation II slopes upward along the 24–26°C isotherms and off the transect to the northwest. Striations III, IV, and V culminate in or are adjacent to a higher level of scattering at the sharp thermal gradient. The upper striation was intense and saturated the sonar system.

The maximum horizontal temperature gradient was approximately 0.015°C/m whereas the maximum vertical temperature gradient was aproximately 3.0°C/m. A plot of the smoothed vertical temperature gradient indicated an area from 50 to 80 m in the thermal boundary water where the gradient was >1°C/m.

The most intense backscattering occurred in this area of greatest temperature change in the vertical field.

Discussion

This acoustic transect provides a rapid, high-resolution view of the distribution of nekton biomass in a complex dynamic area at the edge of the Gulf Stream. In this presentation, we have the ability to resolve the distribution of biomass over 1-m depth and approximately 300-m horizontal intervals. If we had not averaged over 120 pulses at sea, horizontal resolution could be increased to about 20 m (approximately 8 pulses) at the speed we traveled. More traditional biological sampling with towed nets usually integrates over tens of metres in the vertical scale and over kilometre scales in the horizontal. If we used a net sampling strategy of lower spatial resolution of one sample per 10 m of depth in each kilometre, 225 net samples would be needed for the 150 m by 15 km area. To complete this design for daylight periods only would require at least 4 d of net sampling during which the structure would have probably changed. This time frame compares with 2 h 17 min for the acoustic transect. With onboard computer facilities the final results can be displayed either "real time" or shortly after (10–15 min) the completion of a transect. Biological information extracted from net catches often requires months or years of laboratory sample processing.

For the biologist, one logical application of acoustic observations is in conjunction with net sampling. With our acoustic profile, we can design net sampling protocols at sea to test hypotheses on factors governing the small-scale distribution of biomass in the ocean. For example, the location of scatterers suggests an efficient sampling design to test whether each concentration of biological sound scatterers is a distinct taxon or assemblage of species or whether the biological composition of the same band changes when it crosses isotherms or depth levels (Fig. 2). An inherent, but testable assumption in this approach is that the biological structure observed with acoustics delineates biologically meaningful surfaces. Experience with studies of deep scattering layers (Farquhar 1971) and the application of sonar by commercial and recreational fishers certainly suggests that the assumption has some validity.

A second interesting application of acoustics by biologists is to use the sonar information itself to characterize and analyze the biology of complex, dynamic areas such as ocean fronts. Acoustics can be used as a new sensor without nets to view and rationalize biological oceanography at time and space scales possible only with acoustics. To operate at these scales, taxonomic resolution must be sacrificed. Hierarchy theory (Allen et al. 1984; Sugihara et al. 1984; Magnuson et al. 1986) provides a context for such an approach where acoustics can be used both to identify biological surfaces and examine the grain within surfaces at several scales of spatial resolution. Acoustic observation not only can give some estimate of biomass density, but also under certain conditions can yield information on numerical density and size structure of the biota. Pieper and Holliday (1984) have an operating acoustic system, for example, to estimate size structure of zooplankton in situ at sea. Clay (1983) and Ehrenberg et al. (1981) have developed two separate methods to estimate body sizes of nekton acoustically in situ at sea. Such analyses can be accompanied with net or pump sampling to add traditional biological information at lower levels of time and space resolution. Our working hypothesis is that analyses of the acoustic records themselves at levels of biological organization above the species may initially lead to a broader and more innovative comprehension of complex, dynamic areas such as the Gulf Stream front.

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Size Distribution in Periphyton[1]

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When periphytic organisms growing on artificial plants were grouped in logarithmically increasing size classes, patterns in the distribution of biomass were consistent over the season and under different grazing regimes, despite taxonomical changes in the dominant taxa of algae and invertebrates. The amount in each size class was not constant, and instead, certain classes tended to have values consistently lower or higher than the mean. The locations of these peaks and troughs in the size distributions coincided with those observed in lake plankton. Despite these irregularities, the hypothesis that the biomass in logarithmic size classes is constant apparently applies at a crude level, for the biomass of most size classes lay within an order of magnitude of the mean. This rule of thumb also applies to the littoral fish community, for the minnow biomass was approximately that in a periphyton size class.

Lorsque des organismes périphytoniques se développant sur des plantes artificielles sont groupés par ordre de taille croissant sur une échelle logarithmique, on observe des caractéristiques dans la distribution de la biomasse qui se répètent d'une saison à l'autre et dans différents régimes de broutement, en dépit de modifications taxonomiques dans les taxons dominants d'algues et d'invertébrés. Le nombre dans chaque classe de taille n'est pas constant et certaines classes ont plutôt tendance à présenter des valeurs qui sont constamment inférieures ou supérieures à la moyenne. Ces fluctuations dans la répartition en fonction de la taille coïncident avec celles observées dans le plancton lacustre. En dépit de ces irrégularités, l'hypothèse que la biomasse est constante sur une échelle logarithmique s'applique apparemment à un niveau fondamental, car la biomasse dans la plupart des catégories de taille ne s'écarte pas de la moyenne de plus d'un ordre de grandeur. Cette règle empirique s'applique également aux populations de poissons du littoral, car la biomasse de ménés correspond approximativement à celle d'une catégorie de taille de périphyton.

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Size appears to be an important parameter in ecological processes, at both individual and community levels (Peters 1983a). Sheldon et al. (1972) proposed a model for size distribution in pelagic marine communities, where the biomass of organisms, grouped in logarithmically increasing size classes, is roughly constant. This model has been confirmed in some studies of the limnetic plankton (Peters 1983b), but others described peaks and troughs in the distribution (Sprules et al. 1983; Sprules and Munawar 1986; Peters 1985).

This pattern in plankton is apparently based on a constant ratio between the biomass of predator and prey (Kerr 1974). This mechanism may be less important in attached communities, where living forms are more morphologically varied, where there are large amounts of detritus, and where the biota compete more for space. Study of marine benthic communities (Schwinghamer 1981) suggests that the size distribution of lake periphyton could indeed differ from that of plankton. On the other hand, similarity between size distributions in periphyton and in plankton would suggest that size-dependent metabolism and predation are also the main driving forces in attached communities and extend the generality of limnetic allometric models.

To test these hypotheses, I describe the size distribution of algae and invertebrates during the colonization of plastic plants in Lake Memphremagog (Quebec). Experimental manipulation of grazer size (Cattaneo and Kalff 1986) also permits a test of the generality of this distribution in communities dominated by taxonomically different organisms.

Methods

Periphyton communities were grown on plastic aquarium plants ("Hygrophila", R. Hagen, Montreal, Que.) immersed at 50–70 cm depth in MacPherson Bay, in the mesotrophic (total phosphorus = 14 $\mu g \cdot L^{-1}$) central basin of Lake Memphremagog (76°16′W, 45°06′N). A total of 160 plants were placed inside 10 nearly cubical open frames (50 × 50 × 60 cm), each consisting of four 5 × 5 cm posts at the corners of a plywood base and supporting a transparent Plexiglas cover. Grazer size was manipulated by fitting four pairs of cages with nylon cloth of 0.1-, 0.25-, 0.5-, or 2-mm mesh. The remaining two frames remained open and served as controls (Cattaneo and Kalff 1986). Periphyton was sampled after 2 wk (31 July 78) and 6 wk (29 August 78) of colonization. At sampling, two halves of a 660-mL Plexiglas box were closed around two apical leaves ($5.2\ cm^2 \cdot leaf^{-1}$) of five randomly selected plants per box. Water in the boxes was carefully drained through a 0.1-mm-mesh net. The retained material and the periphyton scraped

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[1] Contribution of the Lake Memphremagog Project, Limnology Research Centre.

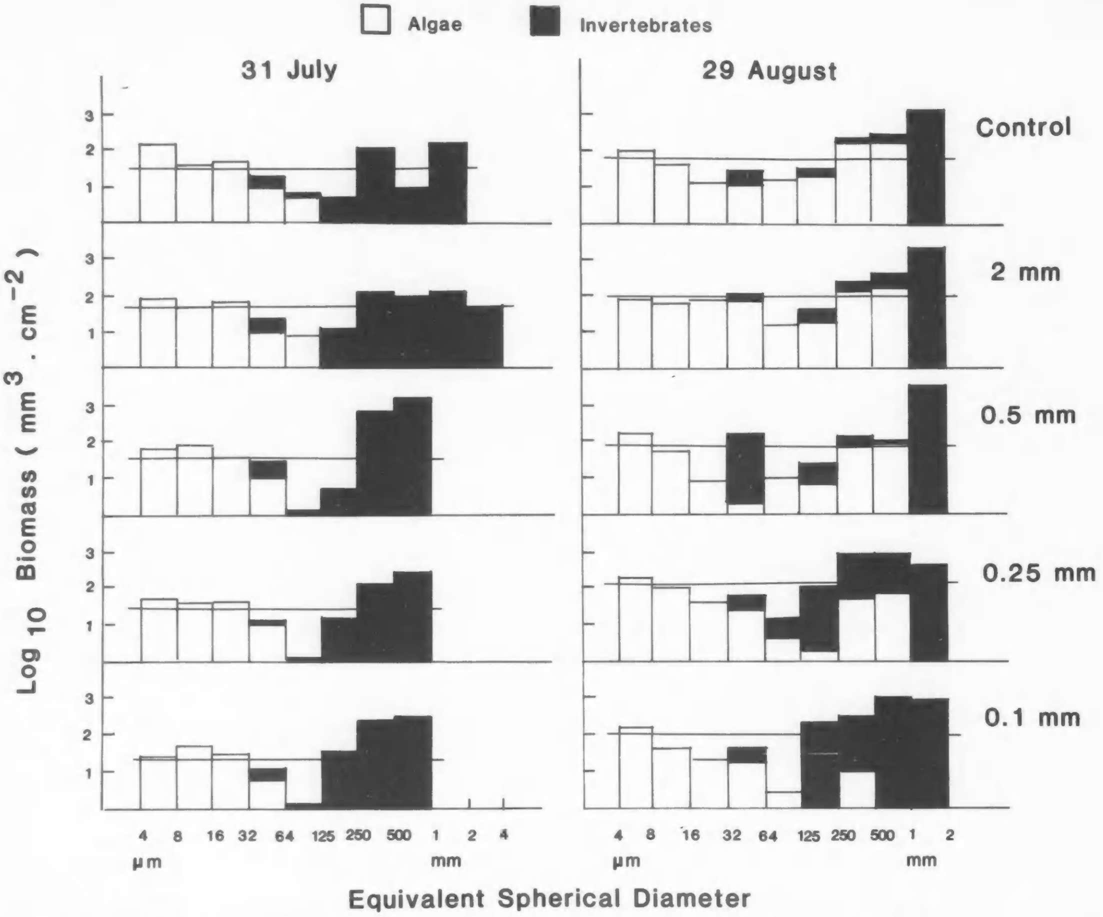

FIG. 1. Size distribution of algae (open bars) and invertebrates (shaded bars) growing on artificial plants in MacPherson Bay, Lake Memphremagog, at two dates during the colonization, in controls and in various treatments in which grazer size was manipulated. Note the change in scale from μm to mm. Horizontal lines represent the geometric means.

from the plastic leaves with a scalpel were fixed in 4% neutral formalin–sucrose solution (Prepas 1978). This procedure minimized periphyton loss and included organisms loosely associated with the substratum, which might otherwise have been lost.

Algae were counted in random fields of a nannoplankton chamber at 400× and 160× magnification. Large filamentous algae, *Coleochaete* spp.colonies, protozoans, rotifers, and nematodes were counted in a Sedgwick–Rafter chamber (100× magnification), whereas *Gloeotrichia* colonies and larger invertebrates were examined under a dissecting microscope at 25× magnification. Cell volumes of algae and protozoans were calculated by approximation to solids of known volume. When colonies or filaments were present, the whole volume was taken as algal size rather than the volume of the single cell. Average weights for each taxon and size class of the main invertebrates were obtained by weighing dried samples (60°C) of each group on a Cahn electrobalance. For snails, weight of the organism without the shell was estimated as 20% of total weight. Dry weights were converted to volume by assuming wet weight = 4 × dry weight and specific gravity = 1. Organism volumes were converted to "equivalent spherical diameters" (ESD) to allow comparison with previous models of size distribution (Sheldon et al. 1972; Sprules et al. 1983).

Results and Discussion

Algae and invertebrates were grouped in logarithmically increasing size classes (Table 1) and the total volume of each class was calculated. The shape of the resulting size distributions appeared similar across communities at different stages of colonization and among different grazer exclusion treatments (Fig. 1). Biomass was not evenly distributed among the size classes; instead, some classes were significantly higher (ESD 4–8 μm; 250–500μm; 0.5–1 mm; 1–2 mm) or lower (ESD 32–64 μm; 64–125 μm; 125–250 μm) than the average value (Wilcoxon rank sign test, *n* = 20).

Although this pattern was relatively consistent, some effects of time of colonization and of size manipulation were evident. The size of both algae and invertebrates was higher after 6 wk of colonization (29 August; average algal size 250 μm^3; inverte-

TABLE 1. Dominant organisms in the various size classes. Sizes are expressed as equivalent spherical diameters (ESD).

ESD	Algae	Invertebrates
4–8 μm	*Achnanthes minutissima* *Gomphonema* sp.	—
8–16 μm	*Cymbella ventricosa* *Eunotia* sp.	—
16–32 μm	*Cymbella* sp. *Cosmarium* sp.	—
32–64 μm	*Synedra ulna* *Oedogonium* sp.	*Carchesium* sp.
64–125 μm	*Coleochaete orbicularis*	Copepod nauplia Nematods
125–250 μm	*Gloeotrichia pisum*	*Chydorus* sp. *Aelosoma* sp.
250–500 μm	*Gloeotrichia pisum*	Small chironomids Small oligochaetes Ostracods
0.5–1 mm	*Gloeotrichia pisum*	Chironomids *Sida crystallina* Oligochaetes
1–2 mm	—	*Amnicola* sp. Large chironomids

brate size 40 μg) than after 2 wk (31 July; average algal size 183 μm^3; invertebrates size 14 μg). This agrees with the hypothesised general trend to larger size with succession (Odum 1969). The manipulation of grazer size produced communities that were very different taxonomically (Cattaneo and Kalff 1986), especially after 6 wk of colonization (29 August). Controls and large-mesh treatments (2 mm) were dominated by the large colonial blue-green *Gloeotrichia* and by snails, which were replaced in small-mesh treatments (0.1 and 0.25 mm) by diatoms and chironomids. Intermediate treatments (0.5 mm) had an intermediate composition. In consequence, the relative contributions of algae and invertebrates to the biomass of some size classes (125–250 μm; 250–500 μm; 0.5–1 mm) were very different in the treatments, while the shape of the size distribution remained remarkably similar (Fig. 1). These results suggest that when periphytic algae become bigger, as it appears to be the case in eutrophy (Cattaneo 1987), grazers may also became bigger.

Sprules et al. (1983) hypothesized a coupling between the size distributions of pelagic and benthic communities in lakes where maxima in one community would coincide with minima in the other. In this study, the size distribution of periphyton was rather similar to that described for lake plankton (Sprules et al. 1983). The minimum appeared at 64–125 μm and the maximum at 0.5–1 mm in both communities. However, in the open water there is another maximum, around 8 μm ESD, which roughly corresponds to phytoplankton. This size class was much less important in periphyton. In fact, the ratio of algae to invertebrate biomasses (average 0.3) was much lower than in plankton (median 1.6; Sprules et al. 1983). This difference may reflect the importance of detritus in the diet of benthic invertebrates (Glowacka et al. 1976). Both periphyton and plankton studies indicate a trough in biomass at 64–125 μm ESD. In the case of periphyton, this cannot represent a gap between two different sampling or counting techniques, since the organisms were counted in the same sample at overlapping magnifications. It could partly result from an underestimation of protozoans, likely to be destroyed or distorted by the fixation (Pennak 1978). However, in unpreserved samples of a lake zoobenthic community, Strayer (1986) also found that the biomass of small organisms (32, 64, and 125 μm ESD) was from one to three orders of magnitude lower than the biomass of larger ones (0.5 and 1 mm ESD). Peters (1983b), measuring the particulate phosphorus of plankton fractions obtained by serial filtrations, also found that some fractions had significantly lower or higher values than expected.

TABLE 2. Biomass, as fresh weight, of the various periphyton size fractions (algae and invertebrates), minnows, and *Myriophyllum* in MacPherson Bay, Lake Memphremagog. Periphyton data represent mean ± SE of values in controls and treatments after 6 wk of colonization (n = 10). Littoral fish biomasses are from (i) Gascon and Leggett (1977) and (ii) Hanson and Leggett (1985). *Myriophyllum* biomass is from C. Duarte (pers. comm.).

	ESD (μm)	Biomass (g·m^{-2})
Periphyton	4–8	0.82±0.14
	8–16	0.32±0.04
	16–32	0.16±0.05
	32–64	0.44±0.10
	64–125	0.07±0.01
	125–250	0.45±0.19
	250–500	1.81±0.47
	500–1 000	3.07±0.84
	1 000–2 000	8.71±2.26
Minnows	8 000–16 000	0.8 (i) 1.5 (ii)
Myriophyllum	16 000–32 000	750

Due to the limitations of the sampling and counting techniques, this periphyton size distribution does not extend to fractions smaller than 4 μm (small algae and bacteria) or bigger than 2 mm (large snails, crayfishes, and larval fishes). However, data on minnow biomass in the same bay have been collected in two different studies (Gascon and Leggett 1977; Hanson and Leggett 1985) and a crude comparison is possible.

For this purpose, the periphyton data, expressed until now as biomass per unit of substratum, were computed as biomass per unit of lake area. This conversion assumes that the communities growing on artificial and natural plants are similar (Cattaneo and Kalff 1978). It also uses a conversion factor for surface to weight of plant (Cattaneo and Kalff 1980) and an estimate of plant biomass in the same bay (C. Duarte, Department of Biology, McGill University, Montreal, Que., pers. comm.). The biomass in the littoral fish fraction (8–16 mm ESD) was surprisingly close to that observed in the various periphyton fractions (Table 2), despite all the assumptions in the calculations. In contrast, macrophytes, like *Myriophyllum* (16–32 mm ESD), had a biomass two to three orders of magnitude higher than fish and periphyton (Table 2). The plankton literature (Kerr 1974; Sheldon et al. 1977; Borgmann 1982) suggests that trophic interactions result in similar biomasses in logarithmically scaled size classes. The similarity in biomass of algae, invertebrates, and fish may therefore reflect tight linkage among these organisms. The disproportionately larger biomass of macrophytes suggests instead that they play a minor role in the trophic relationships of the littoral zone. This is consistent with the widely held view that macrophytes mainly provide a substratum for periphyton rather than food for invertebrates or fish (Sozska 1975).

This study shows that the size structure of the periphyton community is relatively consistent, like that observed in lake plankton, and that it may extend to the fish. Allometric models of community structure and function (Griesbach et al. 1982; Platt 1985; Vezina 1986) may therefore extend beyond the plankton for which they were initially developed.

Acknowledgements

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Influence of Prey Type on Growth of Young Yellow Perch (*Perca flavescens*)

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Confer, J. L., and G. J. Lake. 1987. Influence of prey type on growth of young yellow perch (*Perca flavescens*). Can. J. Fish. Aquat. Sci. 44: 2028–2033.

Growth of age 0+ yellow perch (*Perca flavescens*) fed different zooplankton diets was examined in the laboratory during June and July 1983. Fish were fed an equal mass of diet types that varied with respect to prey species, mean length of the same prey species, and species mixture. Fish consumed all prey within 4 h of feeding. In four trials the ranking of diet type by fish growth was always *Diaptomus sicilis* > Oneida Lake tow sample > *Daphnia* spp. Differences in fish growth frequently varied by 50–300%. These differences suggest that nutritional processes, such as digestion or assimilation, will influence the optimal prey selection for, at least, small zooplanktivorous fish.

La croissance de perchaudes (*Perca flavescens*) d'âge 0+, nourries de divers aliments à base de zooplancton, a été étudiée en laboratoire en juin et juillet 1983. Les poissons recevaient une masse égale de types d'aliments qui variaient en ce qui concerne les espèces proies, la longueur moyenne de la même espèce proie, et la combinaison d'espèces. Les poissons consommaient toutes les proies dans les 4 h qui suivaient la distribution. Dans quatre essais, l'ordre des types d'aliments en fonction de la croissance des poissons était toujours *Diaptomus sicilis* > échantillon prélevé dans le lac Oneida > *Daphnia* spp. Les différences dans la croissance des poissons varient fréquemment de 50 à 300%. Ces différences semblent indiquer que les processus nutritionnels, comme la digestion ou l'assimilation, influent sur le choix de la proie optimale, au moins pour les petits poissons zooplanctivores.

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Models of prey selection by visually feeding zooplanktivores have assumed that all prey have the same qualitative value (e.g. Werner and Hall 1974; Confer and Blades 1975; O'Brien et al. 1976; Eggers 1977; Werner et al. 1983; Wright and O'Brien 1984). These studies have assumed the same efficiency of conversion from mass of prey ingested to fish growth for all prey. Tests of models of prey selection have ranked prey in direct proportion to the mass ingestion rate by starved fish feeding briefly in a swarm (Gibson 1980; Gardner 1981; Mittelbach 1981; Butler and Bence 1984; Wetterer and Bishop 1985; Li et al. 1985). Because handling times are often nearly constant for a wide range of prey sizes (Mittelbach 1981; Mills et al. 1984), all of the above models predict that bigger zooplankton are better. If fish growth on different prey types does not have a constant proportion to the mass ingested, then these models and tests need to be revised.

The quality of zooplankton prey, measured as fish growth per unit weight of prey ingested, could vary for several reasons including the following. The digestible fraction of small invertebrates is highly variable (Windell 1966), and for zooplankton the caloric content varies among species and seasonally within a species (Schindler et al. 1971). The specific dynamic action varies with prey type (Schalles and Wissing 1976). The lipid content of *Daphnia* varies during egg development and the molt cycle (Tessier et al. 1983). The size of the zooplankton could influence the rate of digestion in several ways. Smaller zooplankton have greater surface area per mass which might enhance their digestion by planktivores which ingest prey whole, but greater surface area could reduce the speed or efficiency of digestion due to greater chiton content.

In this study we tested if rations of the same prey weight, but different prey types, promoted equal growth of a young, planktivorous fish. Preceding information on prey quality for planktivorous fish is very limited and conflicting or inconclusive. Differences in growth rates with controlled rations of different diets were detected by Gophen (1980) for fingerling *Sarotherodon salilaeum* but not by LeBrasseur (1969) for juvenile chum salmon (*Oncorhynchus keta*). Field studies of planktivorous fishes have shown changes in growth rates when the fish switched diet types (Mills and Forney 1981; Lemly and Dimmick 1982; Lammens 1982; English 1983; Gumaá and Yassin 1984). However, these field studies could not determine whether the change in growth was due to a change in quality or quantity of prey. Our study was designed as a preliminary survey for qualitative effects of prey on fish growth. Consequently, we used a wide range of zooplankton species.

Methods

Fish — Young yellow perch (*Perca flavescens*) collected from Oneida Lake, New York, as eggs were raised on tow net (153-μm mesh) samples of Oneida Lake zooplankton. Four growth trials were run during June and July of 1983. For each trial, equal numbers of similar-sized fish were transferred to each aquarium where they were again selected for uniform size with odd-sized individuals replaced. For Trials I and II, initial weights were obtained by sacrificing all fish from one aquarium. In Trial III, initial wet weights were obtained on each of these older, hardier fish by mildly sedating them with tricaine methanesulfonate (MS-222). No fish mortality occurred during or shortly after this handling. These initial wet weights of fish from six experimental tanks of Trial III showed no statistical difference (ANOVA; $F = 0.496$, df = 5:90), which suggests that our sorting procedures were satisfactory. Initial and final fish dry weights were obtained after drying at 60°C for 36 h for the first trial and 48 h with larger fish of the later trials. Instantaneous growth rates (r) were computed from the mean initial and final dry weight where $r = (\ln \mathrm{Wt}_i - \ln \mathrm{Wt}_i)\cdot \mathrm{d}^{-1}$.

Experimental prey — Zooplankton species fed to young yellow perch included *Diaptomus sicilis* Forbes, *Epischura lacustris* Forbes, *Mesocyclops edax* (Forbes), *Daphnia pulex* Leydig, *Daphnia galeata* Sars, and *Daphnia magna* Straus. *Diaptomus* and *Epischura* were obtained once or twice weekly from Fayetteville–Green Lake, New York. *Daphnia pulex* and *D. galeata* were collected daily from Oneida Lake, New York, with a 153-μm-mesh net. *Daphnia magna* were from a laboratory culture. All species were maintained in laboratory aquaria on blended spinach strained through 100 μm mesh netting.

Sieves were used to separate the *Daphnia* species into small and large categories. Small *D. pulex* passed through two consecutive 1.00-mm sieves and were retained on a 0.45-mm sieve; large *D. pulex* were retained by a 1.00-mm sieve. Small *D. magna* passed through a 1.80- and then a 1.00-mm sieve and were retained by a 0.45-mm sieve; large *D. magna* were retained by a 1.8-mm sieve.

During Trials I, II, and III, young yellow perch in one of six aquaria were fed one of six diet types (Table 1). For these trials the daily total dry weight of the ration approximated 20% of the fish dry weight. This ration approximates the field ration for young perch in Oneida Lake in years when *Daphnia* are abundant (Mills and Forney 1981). During Trial IIIa, rations of three prey types were provided at $30\%\cdot\mathrm{d}^{-1}$. During all trials the rations were adjusted daily for an assumed instantaneous growth rate by the fish of $0.1\cdot\mathrm{d}^{-1}$. In aquaria receiving 1:1 mixtures, young yellow perch received equal amounts of each of the two prey types. The prey ration was quantified by feeding the fish a known volume of each prey type from a stock culture. Daily, triplicate subsamples of each prey type were taken from each stock culture and poured through a tared No. 10 nitex screen. The screens were dried for 22 h at 60°C before weighing. The

TABLE 1. Composition of diet types for each trial, listing prey that accounted for at least 6% of a diet. Abbreviations: *D. sicilis* and *D. minutus* for the genus *Diaptomus*; *D. pulex*, *D. galeata* and *D. magna* for the genus *Daphnia*; *E.* for *Epischura*; and *M.* for *Mesocyclops*.

Nominal diet type	Species or type mixture	Composition of diet types (%)		
		Trial I	Trial II	Trials III and IIIa
Diaptomus	*D. sicilis*	93	98	100
	D. lacustris	6	2	
Small *Daphnia*	*D. pulex*	85		
	D. galeata	15		
	D. magna		100	100
Large *Daphnia*	*D. pulex*	92		
	D. galeata	8		
	D. magna		100	100
Small *Daphnia* and *Diaptomus*	Small *Daphnia*	50	53	52
	Diaptomus	50	47	48
Large *Daphnia* and *Diaptomus*	Large *Daphnia*	50	55	48
	Diaptomus	50	45	52
Lake tow mixture	*D. pulex*	61	12	25
	D. galeata	37	48	23
	D. minutus		17	23
	M. edax			8

biomass fed to the fish was determined after drying by use of the known volumes of the subsamples. Using this approach the actual dry weight fed to yellow perch was measured 1 d later and never perfectly matched the desired weight ration. Subsequently, each daily ration was adjusted to correct for the preceding deviations. Growth efficiencies for the fish were calculated from the initial and final dry weights of the fish and the sum of the dry weights of the daily rations.

Growth experiments — Trial I began on June 9 and was terminated after 10 d. In this experiment, 19 yellow perch were transferred into each of six 76-L aquaria. Prey composition for all trials is shown in Table 1. Trial II began on June 29 and had six aquaria each with 15 fish. Trial II was run longer than Trial I so that real differences, if they occurred, would be more strongly differentiated. *Daphnia magna* was substituted for *D. pulex*, since the latter became increasingly rare in Oneida Lake. Trials III and IIIa began on July 18 with 10 fish added to each aquarium. These trials were terminated after only 8 d because differences in fish growth among the aquaria were already strikingly apparent.

Upon completion of each experiment, all surviving yellow perch were narcotized with MS-222 and then killed in 10% formalin before drying.

In each trial, all fish receiving the same kind of ration were placed in the same aquarium. Fish from the same aquarium are not independent replicates and are not appropriate for ANOVA. Consequently, tests for the effect of diet type on fish growth compared the ranking of diets by growth rates from one trial with the ranking of diets from the other trials.

Results

Prey size separation — During Trial I, two subsamples of each kind of ration were quantified for size frequency distribution and species composition (Table 1). About 15% of the "*D. pulex*" used during Trial I were *D. galeata*, so that a monospecific diet was not obtained. The mean sizes of small and large *D. pulex* in these subsamples were 1.30 and 1.33 mm and 1.52 and 1.75 mm, respectively. Unfortunately, the size range for large and small *Daphnia* overlapped extensively with each other and the tow diet. This overlap probably obscured any possibility of detecting a potential effect of prey size on growth in Trial I. With this limitation on Trial I, suffice it to say that growth efficiencies varied from 38 to 21% and that the diet rankings were *D. sicilis* > *D. sicilis* and small *Daphnia* > Oneida Lake tow > small *Daphnia* > *D. sicilis* and large *Daphnia* > large *Daphnia*.

During Trials II, III, and IIIa, *D. magna* were used in place of *D. Pulex* (Tables 2 and 3). Five subsamples of each kind of ration were quantified during these trials which showed that the culture of *D. magna* contained no other crustacean. Complete separation of the size ranges for small and large *D. magna* always occurred. For small *D. magna* from these subsamples the range for the mean size was 1.03–1.36 mm. The range of the means for large *D. magna* was 2.17–3.24 mm.

The diet rankings were remarkably consistent throughout this study. In Trials I, II, III, and IIIa, *D. sicilis* always produced the best growth. Given that *D. sicilis* did the best in Trial I, the probability that the same diet would also do best in all the other trials due to random variation can be estimated as 1/108 (i.e. $1/6 \times 1/6 \times 1/3$). In Trials II, III, and IIIa the ranking of diets by fish growth was always *D. sicilis* > lake tow > large *D. magna*. Given that this ranking occurred in the second trial, the probability that the same ranking would occur by chance in both of the next two trials can be estimated as 1/36 (i.e. $(1/3 \times 1/2)(1/3 \times 1/2)$). Pure diets of large or small *Daphnia* spp. produced lower growth than any other pure diets for all trials. Growth with small *D. magna* was the lowest of the six diet types in both trials that used this prey type and appeared to be considerably lower than growth on large *D. magna* taken from the same *Daphnia* culture.

TABLE 2. Growth of young yellow perch fed monospecific and mixed diets of zooplankton at 20% of their estimated dry weight in Trial II. The duration of the experiment was 14 d, and the mean daily dry weight ration was 13.5 mg.

Diet	Mean prey size (mm)	Fish					
		Number of survivors	Mean dry weight (mg) (SE)			Growth	
			Initial	Final	Gain	Instantaneous (r) (×100)	Efficiency (%)
Diaptomus sicilis	0.88	15	41 (2.5)	121 (5.2)	80	7.73	42.3
Large *Daphnia magna* and *Diaptomus sicilis*	1.61	12		106 (5.9)	65	6.80	34.4
Oneida Lake tow	1.23	15		100 (6.8)	59	6.40	31.2
Large *Daphnia magna*	2.34	14		80 (5.6)	39	4.80	20.6
Small *Daphnia magna* and *Diaptomus sicilis*	1.01	15		78 (6.3)	37	4.70	19.5
Small *Daphnia magna*	1.03	15		62 (4.1)	21	3.00	11.1

TABLE 3. Growth of young yellow perch fed monospecific and mixed diets of zooplankton at 20% of their estimated dry weight in Trial III, or 30% of their estimated dry weight in Trial IIIa. The duration of the experiment was 8 d, and 10 of 10 fish survived in all diets.

Diet	Trial	Mean prey size (mm)	Mean prey daily dry weight ration (mg)	Fish				
				Mean dry weight (mg) (SE)			Growth	
				Initial	Final	Gain	Instantaneous (r) (×100)	Efficiency (%)
Diaptomus sicilis	III	1.08	19.2	71.1 (2.1)	115.8 (5.8)	44.7	6.09	29.1
Large *Daphnia magna* and *Diaptomus sicilis*	III	1.78			98.7 (2.5)	27.6	4.10	17.9
Oneida Lake tow	III	1.29			88.8 (2.2)	17.7	2.79	11.5
Small *Daphnia magna* and *Diaphnia magna*	III	1.22			82.7 (2.6)	11.6	1.89	7.5
Large *Daphnia magna*	III	2.48			82.2 (1.4)	11.1	1.81	7.2
Small *daphnia magna*	III	1.36			71.3 (2.6)	0.2	0.04	0.1
Diaptomus sicilis	IIIa	1.08	28.8		141.4 (8.3)	70.3	8.50	30.5
Oneida Lake tow	IIIa	1.29			128.0 (6.1)	56.9	7.30	24.7
Large *Daphnia magna*	IIIa	2.48			112.0 (3.7)	40.9	5.70	17.8

Growth efficiencies within Trials II, III, and IIIa commonly differed by 50–300%, with even larger differences occurring during Trial III. Despite this variation, the ratios for the growth efficiencies for specific diet pairs were relatively constant from trial to trial. As examples, the ratios of the growth efficiencies for Oneida Lake tow samples compared with large *D. magna* were 1.51, 1.59, and 1.39 for Trials II, III, and IIIa, respectively. The ratios for *D. sicilis* compared with the Oneida Lake tow sample were 1.45, 1.36, 2.53, and 1.23 for Trials I, II, III, and IIIa, respectively. Growth efficiencies for the mixed diets were always intermediate to the value for the relevant, pure diets in all six cases. If random factors had affected each aquarium containing all the fish receiving one diet type for one trial, then relationships of the growth efficiencies among the diet types would not have been as consistent from trial to trial.

Differences in fish growth seem particularly striking when the effect of the 10% daily increment in ration is considered. We intended for the daily increment in ration to compensate for fish growth so that fish would be fed a nearly constant percentage of their weight throughout a trial. In fact, we overestimated even the best fish growth, and the daily increment actually exaggerated differences in the ratio of the ration weight to fish weight. Fish that grew less received a higher percentage of their body weight than fish that grew well. At the extreme, on the last day of Trial III the ration of small *D. magna* equalled 54% of the fish weight and was totally consumed within a few hours. Despite eating nearly 50% of their weight each day for the last several days of this trial, growth on a diet of small *D. magna* was negligible. In the same trial, fish on a diet of *D. sicilis* grew by 63% and on the last day of the trial received a relatively smaller ration equal to 33% of the fish weight.

Discussion

The large magnitude of differences in growth, the consistency from trial to trial in the ratio of growth between diet pairs, and the highly consistent ranking of diet types establish that the same mass of different prey types produces differences in growth for young yellow perch. Many studies of prey selection by small, planktivorous fish (see earlier citations) assumed implicitly that equal weight of all prey types provides equal value to the fish. To the contrary, our results show that growth of yellow perch of 24–40 mm was influenced by some qualitative difference among the types of zooplankton provided.

The calorific value and the amount of energy fish expend to capture a prey vary among the prey types. These factors can have a major influence on diet quality for fish (Paloheimo and Dickie 1966). However, for the following reasons, we suggest that these factors are not major contributors to the differences observed in this study.

Reported calories per gram dry weight for *Daphnia* vary widely: 5737 for *Daphnia* spp. from Lake Mendota (Wissing and Hasler 1968) and 5640 for *D. magna* from a laboratory culture (Moshiri and Cummins 1969) to 3633 and 3943 for *Daphnia* with eggs from Lake Mendota (Wissing and Hasler 1968). Our *D. magna* culture was maintained with an abundance of food and most adults had many eggs. Thus, these *D. magna* probably had higher than average calorific values. The calorific value for our *D. magna* can be estimated as 5017 calories·g dry wt^{-1}. This is the average of many values for *Daphnia* with eggs (Richman 1958; Comita and Schindler 1963; Wissing and Hasler 1968; Moshiri and Cummins 1969; Schindler et al. 1971; Lemcke and Lampert 1975) excluding the two lowest values mentioned earlier. Eggless *Daphnia* have lower calorific values. Wissing and Hasler (1968) and Moshiri and Cummins (1969) reported an average reduction in calorific value of 12% comparing concurrent samples of eggless preadults with egg-bearing animals. Using this value, the calorific value for our small *D. magna* can be estimated as 4414. For calanoids the average of 11 values from summer samples (Comita and Schindler 1963; Moshiri and Cummins 1969; Schindler et al. 1971) is 5557. Higher values for calanoids are reported for a large number of observations and are probably real and probably of some importance to fish. However, the estimated differences in calorific values among our daphnid and calanoid diet types were only about 10–20%. The differences in fish growth observed in this study commonly were in the range of 100–300%. This suggests that factors in addition to calorific value affect prey quality for fish.

A greater calorific value for calanoids may be somewhat balanced by a greater cost of capture. *Diaptomus sicilis* was the smallest of the common prey. It was also the most elusive: fish often missed *Diaptomus* when striking at them; the fish seemed to accelerate more when pursuing a *Diaptomus* than when pursuing a *Daphnia*; and the fish often made repeated lunges at a *Diaptomus*, which rarely happened with *Daphnia*. Thus, capture effort per mass of prey ingested was undoubtedly greater for *Diaptomus* than for *Daphnia*. From this perspective, it is surprising that *D. sicilis* always provided the greatest growth.

This study has not determined the nature of the qualitative differences among prey types. It is clear, however, that qualitative differences do exist. Nutritional factors, such as digestive speed and efficiency, assimilation, and specific dynamic action, may be important to prey selection by planktivores. These factors may be particularly important for young fishes with poorly differentiated guts, some of which have prey preferences quite different from those of larger planktivores (Mills et al. 1984).

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Effects of V-Notching on the Lobster, *Homarus americanus*

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No infections were detected in V-notched lobsters, *Homarus americanus*, held for 56 d in the laboratory. During an outbreak of gaffkemia ("red tail") at a commercial lobster pound, 2 of 20 V-notched lobsters died, but 4 of 10 unnotched controls also became infected. Results from challenge experiments with *Aerococcus viridans* (var.) *homari* showed that V-notch wounds healed sufficiently in 24 h to prevent a "red tail" infection. Histological observations of this wound repair of V-notched uropods also demonstrated that within 24 h the wound was sealed by a heavy infiltration of hemocytes.

Aucune infection n'a été relevée chez des homards (*Homarus americanus*) portant l'encoche en V et maintenus en laboratoire pendant 56 j. Au cours d'une épidémie de gaffkyaémie dans un vivier à homards commercial, 2 homards sur 20 portant l'encoche en V sont morts, mais 4 des 10 témoins non marqués ont aussi été infectés. Les résultats des tests menés avec *Aerococcus viridans* (var.) *homari* ont montré que les blessures causées par l'encoche guérissaient suffisamment en 24 h pour empêcher l'infection. Les observations histologiques de la cicatrisation des uropodes marqués avec l'encoche ont aussi démontré qu'en 24 h la blessure était scellée par une forte infiltration d'hémocytes.

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Notching a V in the tails of egg-bearing lobsters, *Homarus americanus*, has been used as a conservation measure to protect Maine's mature female lobsters since 1947 (Anonymous 1947). Prior to then, punching a hole in one of the uropods had been used since 1903. In 1985, an estimated 40 000 lobsters were V-notched by Maine's wardens and fishermen (Botsford et al. 1986). Although the V-notching of ovigerous female lobsters is a standard marking technique in the Maine lobster fishery, only limited research on the effects of this and similar wounding has been done (Stewart and Squires 1968; Stewart et al. 1969; E. W. Spurr, New Hampshire Fish and Game, 34 Bridge St., Concord, NH 03301, unpubl. data).

Numerous studies have been conducted on wound repair in arthropods, but most of those concentrate on insects. A few investigators examined crustaceans, specifically the process of wound repair in penaeid shrimp following marking. Fontaine and Lightner (1973) suggested that the puncture wound created during shrimp tagging caused mortalities, probably from improper healing of the cuticle and subsequent secondary infection. Jewett (1986) also reported tagging mortalities with the use of a floy tag on pandalid shrimp.

The concern that V-notching of lobsters has a similar negative effect due to the deliberate wounding and possible infection has been raised by many individuals in the lobster industry (Laitin 1982). The breach of the lobster integument is essential for the transmission of *Aerococcus viridans* (var.) *homari*, the causative agent of "red tail" disease in lobsters (Stewart et al. 1969). Stewart (1975) stated that the exposure to *A. viridans* (var.) *homari* must come simultaneously with, or soon after, integumental rupture for the pathogen to circumvent the lobster's rapid and effective wound healing mechanism.

I investigated the gross and histopathological aspects of wound healing after V-notching lobsters and monitored infection rates of V-notched lobsters held in the laboratory, in a commercial pound, and in the presence of the pathogen *A. viridans* (var.) *homari*.

Materials and Methods

Female lobsters were obtained on the same day of their capture from a local lobsterman. Carapace length, shell condition, and weight were recorded and both chelae closed with rubber bands. Lobsters were held in tanks supplied with a continuous flow of aerated seawater (6 L/min) from Boothbay Harbor. Ambient temperature ranged from 8 to 18°C over the course of the experiments. All lobsters were held for a minimum of 1 wk before hemolymph samples were taken and shown to be free of *A. viridans* (var.) *homari* prior to the start of an experiment. During the infection monitoring experiment, 30 lobsters were held at the laboratory for 56 d (Groups A and B) and another 30 lobsters were held at a local, commercial lobster pound in floating crates for 31 d (Group C). All lobsters were fed twice per week with available lobster bait. A standard V-notch tool was used to mark the right uropod next to the telson. The tool was immersed in 70% alcohol between each use. This sterilization procedure is practiced by the warden service but not by the industry. During the histological time series experiment a rusty pocketknife, borrowed from a local lobsterman, was used to make half the V-notches in order to compare the wounds created with those of the V-notch tool.

Hemolymph samples were taken with a sterile syringe from the abdominal sinus. Hemolymph smears were examined by the use of Gram stain. Approximately 0.1 mL of hemolymph was used to inoculate sterile trypticase soy broth (TSB). Following this enrichment, isolation of *A. viridans* (var.) *homari* was carried out following the method of Stewart et al. (1966).

Infective cell suspensions for the waterborne challenge experiment were prepared by inoculating TSB with hemolymph removed from an infected lobster. The infected lobster was obtained during an *A. viridans* (var.) *homari* outbreak at a local pound. The culture was grown at 25°C for 24 h and then diluted 1:10 with unfiltered seawater to create the challenge bath. Lobsters were exposed for 10 min in the challenge bath immediately after V-notching (Group D) and 24 h later (Group E). The infective *A. viridans* (var.) *homari* culture was checked for purity and a cell count determined by serial plate count method. A single test lobster was injected intramuscularly with 0.5 mL of the *A. viridans* (var.) *homari* challenge isolate to ascertain its virulence. This isolate had already killed approximately 250 lobsters during the disease episode mentioned above.

V-notched uropods were examined histologically following a protocol similar to Fontaine and Lightner's (1973) where they observed wound repair in brown shrimp, *Penaeus aztecus*. The histological samples (Group F) were fixed in Davidson's fixative for 24 h and then decalcified in Cal-Ex (Fisher Scientific) for 1–3 d depending on the degree of calcification. The tissues were then washed in tap water for 4–6 h, dehydrated through a graded alcohol series, cleared in Hemo De (Fisher Scientific), and embedded in paraffin. Serial sections (6 μm) were prepared and stained with hematoxylin and eosin.

At the termination of each experiment, carapace length, shell condition, and weight were again obtained from each lobster. A second hemolymph sample was also taken to check for *A. viridans* (var.) *homari* infection.

Results

Infection monitoring experiments — In the first experiment, V-notched lobsters (average weight 465 g) were held for 8 wk in laboratory tanks and for 4 wk in a local lobster pound and monitored for mortalities and infections. No mortalities or infections were found in V-notched lobsters held at our laboratory (Groups A and B; Table 1). Further, two V-notched lobsters in Group B "egged out" during the experiment and one of the Group A V-notched lobsters and one of the control lobsters molted.

Fortuitously, an *A. viridans* (var.) *homari* outbreak occurred while Group C lobsters were being held at a local lobster pound. Even with this "natural" challenge, only 4 of 30 lobsters became infected and died, 2 V-notched and 2 unnotched controls (2 × 2 contingency table, $X_c = 2.10$, $p > 0.05$). At the end of the experiment, two more control lobsters (Group C) were found infected with *A. viridans* (var.) *homari* (Table 1). Plate counts taken during the natural outbreak ranged from 10^5 to 10^7cfu/mL with *A. viridans* (var.) *homari* isolated although not enumerated.

Results from the *A. viridans* (var.) *homari* challenge experiment (Table 2) showed that the V-notch heals sufficiently in 24 h to prevent a "red tail" infection (2 × 2 contingency table, $X_c = 9.80$, $p < 0.05$). Nine of 10 lobsters were killed by the 4×10^6 cfu/mL waterborne challenge when immersed immediately after V-notching (Group D). Only 1 of 10 lobsters was killed when given 24 h to repair their wounds before they were challenged (Group E). This lobster and two of the control lobsters (Group E) were probably injured just prior to the

TABLE 1. Infection monitoring of V-notched lobsters.

Group	Location	Initial number	Number dead	Number of dead infected[a]	Number of survivors infected	Observation period (d)	Mean time to death (d)
A	Wet lab[b]	10	0	0	0	56	
Controls		5	0	0	0	56	
B	Aquarium	10	0	0	0	56	
Controls		5	0	0	0	56	
C	Local pound	20	2	2	0	31	21±9
Controls		10	3	2	2	31	15±4

[a] Only *A. viridans* (var.) *homari* infections were detected.

[b] Tank conditions were similar in the wet lab and aquarium. They were in separate buildings.

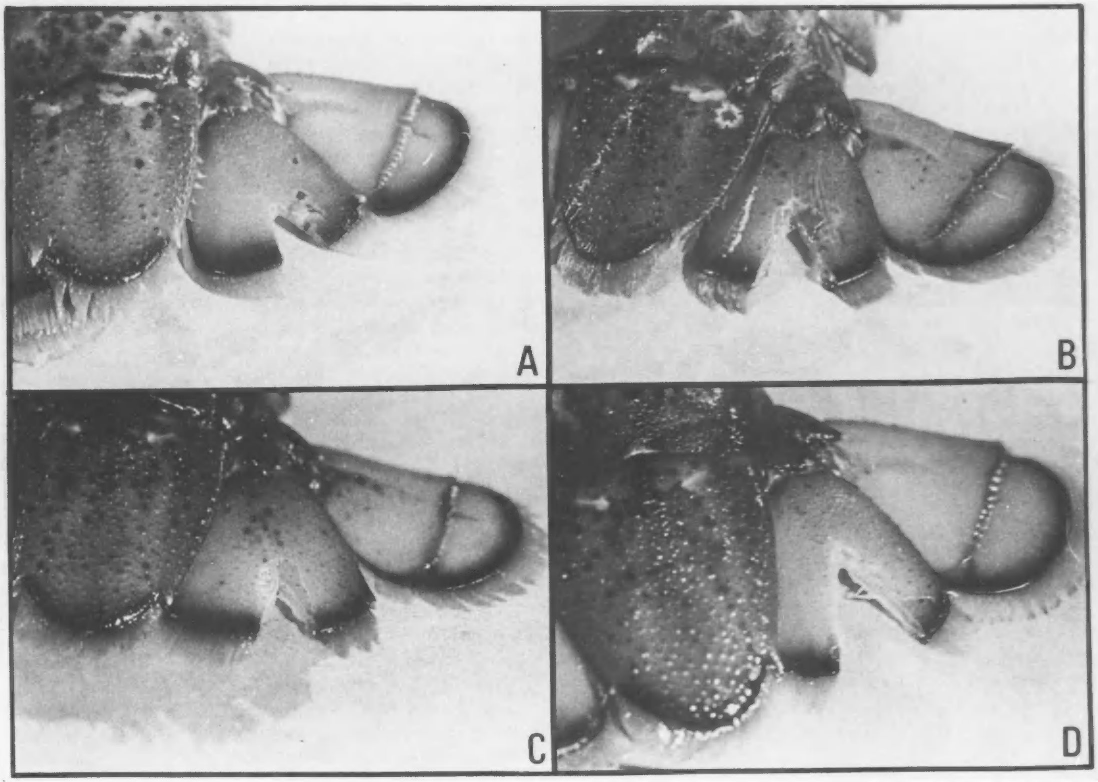

FIG. 1. V-notched uropods cut with a V-notch tool and a pocketknife, respectively. (A and B) Sampled immediately after V-notching (O h); (C and D) sampled at 192 h after V-notching.

challenge. All lobsters that died were heavily infected with *A. viridans* (var.) *homari* and died in the typical 10- to 14-day period except for two of the Group D lobsters that died after 20 and 23 d. The single lobster injected intramuscularly with 2×10^6 cells died after 8 d, confirming the pathogenic nature of the *A. viridans* (var.) *homari* isolate.

Gross and histological observations — The gross appearance of the V-notch wound initially was the translucent pink color of the exposed muscle and connective tissue (Fig. 1A and 1B). Over the first 24 h the wound color darkened slightly and the tissue became more opaque. At 96 h the pink color had changed to a light brown and by 192 h a dark brown color (Fig. 1C and 1D). From the earlier infection monitoring experiment, it was noted that by 2 wk the V-notch wound had completely blackened and this persisted throughout the study. The one specimen (Group A) that molted had the normal carapace coloration in the V-notch wound site.

The histological observations of the healing of the V-notch showed a progressive hemocytic infiltration of the damaged muscle tissue adjacent to the wound over the first 24 h (Fig. 2A–2D). At 12 h the whorling of fusiform hemocytes could be seen forming the beginnings of foci or nodules. Between 24 and

TABLE 2. Waterborne challenge of V-notched lobsters, *H. americanus*, with *Aerococcus viridans* (var.) *homari*.

Group	Location	Initial number	Number dead	Number of dead infected	Number of survivors infected	Observation period (d)	Mean time to death (d)
D[a]	Aquarium	10	9	9	0	25	14.7±3
Controls		5	0	0	0	25	
E[b]	Wet lab	10	1	1	0	25	12.0±0
Controls		5	2	2	0	25	12.5±0.5

[a] Lobsters in Group D were immersed immediately after V-notching in a challenge bath containing 4×10^6 cfu/mL *A. viridans* (var.) *homari*. Immersion time was 10 min.

[b] Lobsters in Group E were immersed 24 h after V-notching under the same conditions as Group D.

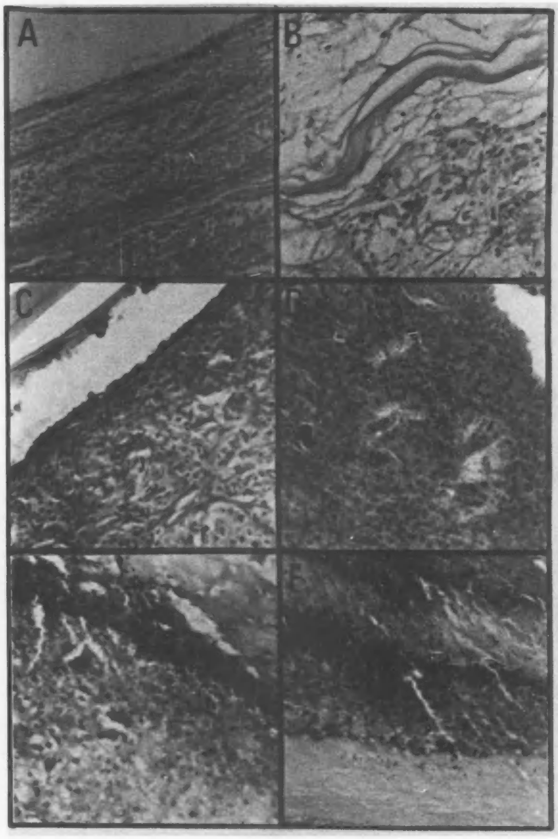

FIG. 2. Progressive hemocytic infiltration of the wound site at (A)0, (B) 6, (C) 12, and (D) 24 h, (E) formation of melanin band at 48 h after V-notching; (F) wound edge after 25 d: the layers of hemocytes appear necrotic, the cuticle has started to be formed by epidermal cells, and melanin bands are still apparent. Hematoxylin and eosin; ×200.

48 h, several layers of hemocytes had formed with a distinct band of golden brown pigment in the middle, presumably melanin (Fig. 2E). The periphery of necrotic hemocytes seemed to be sloughing off. By 96 h the layers of hemocytes were more compact and the cells all fusiform. The encapsulation of necrotic tissue or foreign material could be seen in the many melanized nodules within the layers and also internal to the hemocyte layers. By 192 h, epidermal cells could be seen basal to the hemocytic layers. At 288 h the nuclei in the outer layers of hemocytes were mostly pycnotic. There were still many phagocytes in the adjacent connective tissue. Finally, after 25 d (600 h) the layers of compacted hemocytes all appeared necrotic, the cuticle had begun to be formed underneath these layers by the epidermal cells, and the numbers of phagocytes in the nearby connective tissue had been greatly reduced (Fig. 2F).

No apparent differences were seen histologically when V-notches made with the V-notch tool and a lobsterman's knife were compared. Wounds made by the knife were larger than those made by the V-notch tool, yet both healed at the same rate. The water temperature during this experiment was 11–13°C.

Discussion

In the present study, three experiments were conducted on the effects of V-notching on *H. americanus*. The first experiment involved holding V-notched lobsters under laboratory conditions and in the more stressful environment of a commercial lobster pound. A similar experiment was carried out by E. W. Spurr (unpubl. data), who held V-notched lobsters in cages off the coast of Rye, NH. The only differences were the notches, which were placed in the telson as opposed to the uropod, and the observations, which took place during weekly dive trips. No V-notch-induced mortalities were observed during the New Hampshire study. Of the four lobsters that died during a gaffkemia outbreak at a local lobster pound, two were V-notched and two were unnotched controls. Although the lobsters were checked beforehand for injuries, small wounds in the two controls may have made them as susceptible to infection as the V-notches in the other two fatalities. So even under the more detrimental conditions of a gaffkemia outbreak, the marking of lobsters by V-notching made them no more susceptible to disease than unnotched control lobsters.

Some of the earlier work by Stewart et al. (1969) was repeated to demonstrate the rapid wound healing mechanism of *H. americanus*. Instead of wounding the ventral abdominal membrane as they had done, the deliberate wound tested was the V-notch in the uropod and the exposure time was reduced from 24 h to a 10-min immersion. The results of the two studies were quite similar. They lost 8 of 10 lobsters wounded immediately prior to exposure to *A. viridans* (var.) *homari*, while 9 of 10 from Group D were lost to gaffkemia in the present study. In both studies, only 1 of 10 lobsters (Group E) was lost to "red tail" when wounded 24 h prior to the *A. viridans* (var.) *homari* challenge. Admittedly, lobsters exposed to high doses of *A. viridans* (var.) *homari* immediately after V-notching did die during this in vitro challenge. But the chances of freshly V-notched lobsters being exposed to another infected lobster in the wild are low (Vachon et al. 1981). The rapid healing of the V-notch further reduces the possibility of the V-notch lobster becoming infected.

The wound healing process in *H. americanus* closely follows that found in brown shrimp (Fontaine and Lightner 1973). Rapid hemocytic infiltration seals the wound opening within the first 24 h. Subsequently, multiple layers of hemocytes are laid down and melanin bands are formed within these layers. Foreign material and necrotic tissue are encapsulated by fusiform hemocytes which eventually become melanized. Phagocytes are evident later clearing tissue debris. Epidermal cells slowly develop basal to the compacted hemocyte layers with the formation of the cuticle soon after.

In summary, the chances of V-notched lobsters becoming infected, particularly by *A. viridans* (var.) *homari*, seem to be minimal based on the initial work cited here. The present study has shown that the V-notch wound heals rapidly and that within 24 h, V-notched lobsters can resist artificial and natural challenges by *A. viridans* (var.) *homari*. Histological observations further support the lobster's consistent wound sealing mechanism.

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